YALE UNIVERSITY LIBRARY
3 9002 06584 6504

SERUM COMPLEMENT AND PRECIPITATING ANTIBODY IN BYSSINOSIS

GEOFFREY P. KANE

1971

MUDD
LIBRARY
Medical

YALE

MEDICAL LIBRARY

Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

https://archive.org/details/serumcomplementp00kane

SERUM COMPLEMENT AND PRECIPITATING ANTIBODY IN BYSSINOSIS

Including a Review of the Literature on Byssinosis with Special Reference to Nosology and Pathogenesis

Geoffrey P. Kane

April 1, 1971

YALE MEDICAL
NOV 1971
LIBRARY

Summary

Twenty active and retired Spanish hemp workers were exposed to airborne hemp dust in small hemp processing plants. Some experienced chest tightness and demonstrated decreases of $FEV_{1.0}$. No significant change in serum C'3 levels, measured by an immunoassay technique specific for human β_{1C}/β_{1A}-globulin, was associated with these manifestations of the acute dust reaction of byssinosis. A small number of sera tested by double gel diffusion did not demonstrate precipitating antibody to crude antigens prepared from hemp dust and from cotton bracts. Information pertinent to the pathogenesis of the acute dust reaction and the chronic lung disease of byssinosis is reviewed and used to establish the relevance of the two serological studies described and to structure the conclusions which may be drawn from them. Preliminary remarks use the literature on byssinosis to document the impression that evolution of the diagnostic criteria for that disease has markedly altered its epidemiology, and done so without investigators of the disease being explicitly aware of the process.

To My Parents

Preconceived ideas are like searchlights which illumine the path of the experimentor and serve him as a guide to interrogate nature. They become a danger only if he transforms them into fixed ideas—this is why I should like to see these profound words inscribed on the threshold of all the temples of science: "The greatest derangement of the mind is to believe in something because one wishes it to be so." --Louis Pasteur, in a speech to the French Academy of Medicine, July 18, 1876 (translation by Rene J. Dubos).

Contents

List of Illustrations i

List of Tables ii

Introduction iii

The Occupational Disease 1

The Pathogenesis of Byssinosis 15

1. Acute dust reaction 15

a. Active substance 19

b. Mode of action 27

2. Chronic lung disease 43

3. Summary and comment on pathogenesis 53

Serologic Study: Rationale and Goals 55

1. Serum complement 55

a. Technical considerations 55

b. Serum complement in human disease 60

c. Complement and byssinosis 63

d. Goals and limitations 64

2. Precipitating antibody 67

Methods 70

Results 83

1. Serum complement 83

a. Staff controls 83

b. Hemp workers 83

c. Reproducibility of C'3 measurements 91

2. Precipitating antibody 91

Discussion 92

Conclusion 95

References 96

Appendix (Questionnaire) 116

List of Illustrations

Figures I-III	Callosa de Segura	73
Figure IV	Espadador	75
Figure V	Rastrillador	75
Figure VI	Hyland IMMUNO-PLATE	80

(Figures I-V were reproduced from color transparencies taken by the writer; Figure VI was taken by Mr. J. Holt.)

List of Tables

I Serum C'3 Levels of Research Staff Members Before and After Hemp Dust Exposure 84

II Summary of Selected Questionnaire Responses of Hemp Workers and Ex-Workers with Symptoms, $FEV_{1.0}$, and C'3 Associated with Hemp Dust Exposure 85

III Serum C'3 Levels of Hemp Workers on a Monday Spent Away from Hemp Dust 90

Introduction

This paper is concerned chiefly with the pathogenesis of byssinosis. A comprehensive review of related literature precedes a presentation of research material dealing with delimited aspects of that topic. The review emphasizes observations (i.e. evidence) rather than interpretations of them. The writer has tried to make obvious any statements of his own interpretations of the material of others. To avoid promoting discontinuity in readers' thoughts, some minor points of discussion have been located with their background information in the review section. The preliminary section (The Occupational Disease) presents an explanation which seems, to the writer, necessary to reconcile certain inconsistencies among assumptions about byssinosis to be found in the literature.

Although the data presented were obtained in 1967-68, the general literature review and the information related to the research described have been made as up to date as possible. Recent developments have had little effect on the research itself, except to place the complement study on firmer theoretical ground and to eliminate the necessity of thinking that the complement system could be involved in Type I allergic mechanisms.

Readers should be aware of two biases in the writer's presentation. A simplified concept of asthmatic disease has been used; some readers might be more comfortable had the term

"allergic bronchial asthma" been used in conjunction with some of the material discussed. No mention is made of byssinosis grade III, a classification used intermittently to designate byssinotic survey subjects manifesting severe respiratory impairment, because (1) the use of the term has not been universal, (2) its use expresses pathogenetic assumptions which may not be accurate, and (3) by taking the alternative approach of applying the name of the respiratory syndrome which chronically diseased byssinotics share with many others, there are an increasing number of therapeutic considerations which come to mind.

Acknowledgments. The writer is indebted to many individuals for assistance at various stages of this project. Drs. A. Bouhuys, R. Acheson, and W. Meigs have been particularly helpful. The writer would also like to mention Drs. B. Waksman, F. Kantor, H. Treffers, K. van de Woestijne, J. Douglas, V. Popa, C. Carrington, R. Schilling, A. Barbero, and J. Beloff. J. van Wayenburg, C. Toren, P. Ridgway, G. Cohen, Y. Park, and the staffs of Dr. Kantor's lab and of the WHO Serum Bank all provided some form of technical assistance. Assistance with printed materials was provided by the staff of the Yale Medical Library and by Mrs. M. Bruch and Mrs. D. Piscitelli of Dr. Bouhuy's staff. Mrs. I. Diasio translated the German article by Wuthrich. The writer's wife, Suzanne, typed the bulk of this manuscript. Drs. G. Wyshak, C. White, and, especially, S. Bayard advised on the statistical analysis.

The studies reported in this paper were supported by a grant from the U.S.P.H.S. (UI-00435) to Dr. A. Bouhuys, and by a student fellowship of the John B. Pierce Foundation Laboratory.

The Occupational Disease

Byssinosis* is a respiratory disease associated with the inhalation of organic dust raised in the early stages of processing certain textile fibers. It has been described in cotton, hemp, and flax workers.[8-10] Respiratory problems in sisal, esparto grass, and jute workers may be related.[10-13] Affected workers initially experience chest tightness, cough and shortness of breath at the beginning of the work week. Later, their symptoms may extend beyond the beginning of the week. Eventually, productive cough, effort intolerance, and dyspnea may mark incapacitating, irreversible respiratory impairment.[2,3,14] The natural history of byssinosis** therefore includes both transient, or acute, and permanent, or chronic, manifestations of disease. Other names for the

*Proust introduced this term in 1877. The problems he named with it had been recognized for some time. Ramazzini's 1713 treatise on occupational diseases includes a chapter on "Diseases of dressers of flax and hemp and carders of silk rolls".[1] This and other early descriptions of respiratory problems in textile workers, as well as the origin of the term byssinosis, have been amply reviewed.[2-7] The pieces by Carminita et al.[3] and Kovats and Bugyi[5] provide good accounts of the complex of dust-related problems from which byssinosis, as defined in the text above, has been differentiated.

**Readers unfamiliar with the industrial environments where byssinosis develops are referred to the descriptions of cotton processing by Carminita et al.[3] and of flax processing by Bouhuys et al.[17] and Elwood et al.[18] Hemp processing is briefly described in the Methods section of this paper and in more detail by Barbero and Flores[15] and Bouhuys et al.[16]

disease, such as "strippers' asthma"*, which are rarely used today, reflected these manifestations. The name byssinosis is derived from a Greek word for fine thread, flax, or cotton and reflects the necessary cause of the disease – textile dust. Since most authors, pending more specific identification, seem to regard the dust itself as an agent of disease, byssinosis may be thought of as a disease of known etiology.

Diseases are nothing more than useful abstractions of groups of sick people. Disease categories tend to be dynamic and themselves change over time as the groups they represent shrink or grow, are combined with others or split apart. The preceding definition of byssinosis was formulated to introduce the disease and to be compatible with most definitions to be found in the old and new literature. It contains statements about groups presently excluded from the disease category byssinosis, but there is no indication of evolution of the disease, which might be expected in a definition representative of all the concepts of a disease held within a fairly long time span. The writer believes that changes have taken place in byssinosis and that there is an explanation for why descriptions of the disease have not appreciably changed. Since there does not seem to be

*In the cotton industry the men who perform the dusty operations of removing dirt from and sharpening the teeth of carding devices are known as strippers and grinders.[3]

a general awareness of these changes and because an understanding of them puts the literature on byssinosis in clearer perspective, this first section of the present paper examines them and a number of the reasons behind them. An attempt has been made to incorporate sufficient detail to provide historical background for this study.

The boundaries of the groups of sick people represented by a disease are defined by diagnostic criteria. These explicit or implicit standards regulate the composition of both the identified and the potentially identifiable portions of the group of people who may be said to have the disease. The most obvious changes in byssinosis can be traced to changes in diagnostic criteria.

Many recent studies base the diagnosis of byssinosis solely upon textile workers' own reports of having experienced the acute symptoms of byssinosis. Several[16,19-26] follow the example of Schilling et al.[27] who considered this history quite distinct and documented its usefulness as a diagnostic criterion in population studies by finding very little observer disagreement in its use. Schilling and his group further classified workers with byssinosis as Grade I if they reported chest tightness and/or breathlessness on Mondays only or as Grade II if they reported chest tightness and/or breathlessness on Mondays and other days. Roach and Schilling[28] later added a Grade ½ to designate "occasional chest tightness on Mondays, or mild

symptoms such as irritation of the respiratory tract on Mondays". To confirm the functional significance of subjectively perceived ventilatory impairment, tests of lung function have at times been introduced as further diagnostic criteria. The measurement of a decrease in ventilatory capacity in the course of a day's work has been used both along with reports of acute symptoms[29] and independently[17,30] to diagnose byssinosis. Because changes in lung function during dust exposure has been a common finding in workers who do not give a history of acute symptoms,[20,21,31-33] it has been urged that such changes be considered diagnostic in order to increase the objectivity and the sensitivity of diagnosis.[14,21,34] Of note is that all of these presently accepted criteria, whether lung function tests are included or not, depend upon the detection of acute manifestations of disease.

Carminita et al.[3] quote (P. 47; pp. 43-44) diagnostic criteria for byssinosis promulgated in the nineteen-thirties:

> ...requisites for recognizing beginning pneumonopathy due to cotton dust: (1) Exposure to the dust for a more or less long period of time; (2) dry cough with scanty sputum, thoracic pain; (3) slight notes of pulmonary resonance; (4) weakening of thoraco-vocal-tactile fremitus and of the vesicular murmur; (5) general condition good; (6) absence of fever or rarely a slight increase in temperature; (7) altered respiratory functions (low spirometric values) which provoke notable sufferings in the patient in contrast to the scanty physical findings; (8) presence in the sputum of the elements composing the inhaled dust and absence of the various microorganisms; and (9) lessening of the lesion on removal of the patient from the working atmosphere and tendency toward cure especially in the more recent cases. (Caso, 1932)

> Chronic bronchitis, complicated by emphysema in its later stages and by asthma in some cases, has been unduly prevalent in cardroom operatives... The symptoms and signs of the disease as revealed by physical examination do not differ from those of bronchitis and emphysema as seen in the general population, but the clinical and industrial histories, taken in conjunction with the condition found on physical examination, are sufficiently characteristic to enable a properly constituted Medical Board to arrive at a definite conclusion that the disease is industrial in origin and due to the inhalation of cotton dust... byssinosis... (Report of the Departmental Committee on Compensation for Card Room Workers, 1939)

These clinical criteria depend, in large part, on symptoms and signs of disease which are manifestations of irreversible anatomical change and are therefore among the chronic manifestations of byssinosis. Epidemiologic studies of the morbidity and mortality of cotton workers by Hill,[35] Harvey (cited by Schilling[36]), and Schilling and Goodman[37], done on records compiled for various segments of time between 1921 and 1936, were sensitive only to evidence of severe disease. They examined death rates from respiratory disease and work absences caused by respiratory diseases.

The writer contends that prior to around 1950, the only workers generally thought to have byssinosis were those with chronic manifestations of the disease. The diagnostic criteria in use clinically and epidemiologically excluded less diseased workers from the group of identifiable byssinotics. This is consistent with the belief during that period that byssinosis took ten years to develop.[38] The definition and natural history of the disease recorded then was the same as it is today and included early symptoms

since the histories of byssinotic workers, told after they had been identified by evidence of chronic disease, recounted all their own earlier respiratory difficulties. The transient symptoms occurring in advance of chronic manifestations of disease seem to have been regarded as a curiosity or a nuisance.* They were perhaps sometimes viewed as a prodrome of the disease, but diagnoses were not based on them alone. Workers with only that manifestation of byssinosis remained outside the disease category until the old diagnostic criteria for byssinosis were replaced.

In the late nineteen-forties and the early nineteen-fifties, when field surveys were introduced to the study of byssinosis,[36,39] the acute manifestations of the disease became important diagnostically. There was a need for diagnostic criteria which could be applied uniformly over large numbers of people seen at their place of work. The only characteristics of byssinotics which distinguished them from other people with chronic lung disease were their occupational histories of exposure to textile dust and their reports of having experienced chest tightness on Mondays.

*One early investigator, who had extensive personal contact with the respiratory problems of cotton workers as an inspector of factories, was exceptional in giving some serious attention to these symptoms. Collis' description of the progressive effects of dust inhalation, published prior to 1910, included a "stage of irritation" consisting of non-disabling chest tightness for one or two days on returning to work after a short absence.[3] Collis counted the men in this stage[2] as well as in stages of "temporary disablement or incapacity" and "total disablement or incapacity".[3]

Occupational histories could be of no value differentiating byssinotics from other workers in the same dusty location; so, in view of the remarkable constancy with which the symptom had appeared in the histories of previously identified byssinotics, chest tightness on Mondays was selected as a single diagnostic criterion.[27] It was found that this criterion could be conveniently and consistently applied and it was widely adopted as field surveys of textile workers proliferated.

With acceptance of the new diagnostic criterion for byssinosis the boundary of the group of people with the disease was redefined. The measure of the presence or absence of the disease had been shifted from chronic manifestations of the disease to an acute manifestation, so it might be expected that some of the resulting changes in the composition of that group would not be subtle. There would likely be more people in the new group. On the average, they would be less diseased. Every worker who had experienced acute symptoms of the disease would not necessarily progress to the chronic stage, so byssinosis could no longer be categorically described as a chronic or disabling disease. What had been a pre-disease prodromal period would become part of the formal course of the disease.

The writer has found no evidence in the literature that investigators were aware such consequences would result from a change in diagnostic criteria. None of the above alterations of the disease byssinosis seem to have

been anticipated. In fact, when evidence of them was found, it was met with puzzlement rather than insight. In 1950, Schilling[36] seemed to have difficulty reconciling his impression that steadily improving dust control in British cotton mills had reduced the incidence and severity of byssinosis with the fact that high incidences of byssinosis had recently been found by two of the first field surveys. Almost as an afterthought he added that fewer men than previously were becoming disabled. That last datum might have been able to provide an estimate of incidence which could be compared with previous estimates, but the incidence rates measured by the field surveys pertained to a different byssinosis. As a standard method of studying byssinosis emerged, incorporating historical diagnostic criteria and a field survey approach,[8] a consistent body of information was accumulated. An interesting sign of the gradual acceptance of it as "byssinosis" is to be found in Schilling's 1956 review.[2] This article seems to be one of the most widely cited papers on byssinosis and to have been the source for several other investigators of the graded criteria based on acute disease manifestations. It begins with a brief historical introduction to "a chronic respiratory disease", byssinosis.

A persistent problem deriving from the establishment of new diagnostic criteria and the subsequent modification of them with lung function testing, is related to the shortening of the duration of dust exposure necessary to

produce "byssinosis". When chronic disease evidence characterized the disease, the onset was after about ten years. In 1956, Monday chest tightness was anticipated after "some years" exposure.[2] Since that time byssinosis has been diagnosed in numerous workers exposed to textile dust less than two years,[17] less than one year,[21] or only a few months.[40] Investigators have also demonstrated that the manifestations of byssinosis may occur in non-textile workers at the time of their first exposure to textile dust.[16,41]

While, of late, considerable emphasis has been placed by some investigators on the rapidity with which manifestations of byssinosis may occur, the group of researchers headed by Taylor continues to assume that byssinosis has an appreciable induction time.[42,43] In a recent article, Bouhuys et al.[44] mentioned disagreement with regard to the duration of dust exposure necessary to produce disease manifestations diagnostic for byssinosis and attributed the disagreement to varying uses of the term byssinosis. Yet Taylor's group accepts the same diagnostic criteria for the disease as most other contemporary investigators (specifically, those in Schilling et al.[19]).

In the writer's opinion, and in keeping with the general framework of disease definition assumed in this

paper, the controversy cited is more a matter of disagreement concerning the characteristics of one group of people (as defined by a shared set of diagnostic criteria) than a matter of differing applications of the same term to two groups of people having different characteristics. It might further the pathogenetic understanding of byssinosis if, as more is learned about host factors in the disease, close attention is paid to the possible presence of and significance of a spectrum of onset times.

Within the range of arbitrariness permitted by biological considerations, seemingly the most important factor regulating the nomenclature and definition of diseases is the usefulness of the resulting categories.[45] Each of the two sets of diagnostic criteria for byssinosis which have been under discussion have shown relative stability over time. The durations of their acceptance may then be testimony to their usefulness. The remainder of this section presents practical implications of these criteria which probably promoted their stability. The material adds another dimension to the

consideration of the evolution of byssinosis and should provide further historical information to readers unfamiliar with the disease.

The diagnostic emphasis, in the first half of this century, on chronic manifestations of byssinosis may well have facilitated proving the existence and significance of the disease. In the United Kingdom, trade union complaints had prompted investigation into the health of cotton workers prior to 1910.[35] In these investigations, workers with chronic lung disease should have been easy to identify. The impaired work effectiveness of those identified and their sometimes tragic degree of impairment probably helped motivate attempts to deal with the occupational disease. That attempts were made indicates the early studies had impact. Efforts at primary disease prevention through dust control began in 1912. The cotton industry and the Shirley Institute of the Cotton Research Association maintained these efforts as further investigations continued to find disease. The accumulated documentation of a disabling chronic occupational disease in British cotton workers led to appeasing legislative action in 1940. Compensation benefits were provided for workmen with twenty or more years exposure to cotton dust who were dead of, or totally and permanently incapacitated by, respiratory disease.[46,*] The fact that the early criteria

*Benefits have subsequently been extended to women and to the partially disabled. The writer thinks the minimum length of exposure for eligibility has been reduced from twenty years to ten.

were displaced rather than discarded suggests there was satisfaction with them, suggesting that these social responses to research findings reinforced the approach of investigators.

The requirement of the field survey method for a reproducible measure of the presence of byssinosis led to general acceptance of a criterion which happens to identify byssinotics relatively early in the course of their disease. This seems to have been an adaptive selection for reasons beyond its immediate usefulness.

The primary prevention of byssinosis did not prove to be the easy task which had been anticipated by early investigators. In spite of steady improvements in dust control, there continues to be an incidence of the disease. A specific disease-producing substance or substances have not yet been identified in the dusts, much less removed. There is no specific therapy for any stage of the disease. It has been known clinically for some time that early respiratory impairment in textile workers tends not to deteriorate if the workers avoid the dust. While there is as yet no epidemiologic evidence of the extent or success of such efforts, the secondary prevention of disabling disease seems the most realistic means of mitigating the respiratory effects of textile dusts at present. A means of making early diagnoses is a prerequisite for secondary prevention. The usefulness of the present diagnostic criterion is considerably enhanced by the fact that it promotes early diagnosis. The set of

graded historical diagnostic criteria are supplemented by a recently proposed system for the functional grading of byssinosis. The system is based on a widely used lung function test (forced expiratory volume in one second, $FEV_{1.0}$) and includes concrete recommendations for the protection of workers in each grade.[44]

The usefulness of diagnostic criteria in furthering the interests and preferences of investigators might also be expected to affect the length of time the criteria remain unchanged, since investigators control the definitions in use at least in their own work. Early investigators seemed intent on recording the late manifestations of the disease. Prausnitz'[47] summary of the clinical studies by Collis (1908) and the Departmental Committee on Dust in Cardrooms in the Cotton Industry (1932) indicates that they gave careful attention to the appearance of chronically diseased workers and to studies which might help assess the severity of their disease such as chest radiographs, measurement of chest expansion, or autopsies. Prausnitz' own clinical section includes lung function studies which serve to confirm serious respiratory impairment. Current investigators seem intrigued by the pathogenesis of the disease. Their efforts to advance the understanding of it have focused on the most characteristic aspect of byssinosis, the acute dust reaction. The short time course (about twelve hours) of acute dust reactions makes them convenient to study. However, these reactions are self-limited and there is as yet no benign means of

gaining access to the site where the relevant cellular and molecular mechanisms occur. There is much to be learned about the pathogenesis of byssinosis, which suggests that the present approach to the disease will obtain for some time.

The Pathogenesis of Byssinosis

Acute dust reactions and chronic respiratory problems have seemed so obviously associated in textile workers that they are regarded as aspects of the same disease. Substantiating this assumption is the recent survey finding, in a group of older and retired hemp workers, that chronic respiratory symptoms are more prevalent among those who report having experienced Monday symptoms in the past.[25] While the acute and chronic problems do seem related epidemiologically, there is no evidence that they are both consequences of the same disease mechanism. To avoid implications which tax the applicability of present knowledge, the following review of evidence concerning disease mechanisms in byssinosis will treat the acute and chronic aspects of the disease separately.

1. Acute dust reaction

The symptoms (chest tightness, cough, shortness of breath) and signs (occasionally diffuse wheezes and rhonchi) of the acute byssinotic reaction to cotton, hemp, and flax dusts suggest an impairment of ventilation. These clinical manifestations are quite similar to those of attacks of bronchial asthma.[15,29] The reaction subsides in a few hours once away from the dust. The cough sometimes present in acute dust reactions is not productive of conspicuous amounts of sputum. Examination of sputum specimens from byssinotics

has revealed mixed microbial flora.[47] The presence of eosinophils in sputum has been described in only one study[17] and it is not considered typical of the disease. Blood eosinophilia has not been found.[17,47] Leukocytosis was found in four byssinotic flax workers four to six hours after the start of work on a Monday and a Wednesday.[17] Fever is not considered characteristic of the acute dust reaction, although confusion has sometimes arisen because there have been reports of temperature elevation and malaise in workers coming in contact with textile dust for the very first time. These reports are now grouped together as "mill fever".[3,8]

Physiologic tests have been used to confirm the presence of, and to characterize, the reduction of ventilatory capacity which is the chief manifestation of the acute dust reaction. Pulmonary function studies during acute reactions, when compared to pre-reaction studies in the same subject, have shown increased residual volume (and/or functional residual capacity),[17,29,48-49] unchanged or increased total lung capacity,[48-49] increased ratio of residual volume to total lung capacity,[17,48] decreased vital capacity,[17,29,33,48-50] decreased timed or forced expiratory volume ($FEV_{0.75}$ or $FEV_{1.0}$),[12,16,17,19-22,24,29-33,48,50] decreased maximal mid-expiratory flow rate,[21,31,48] decreased maximum breathing capacity (usually measured indirectly as forty times $FEV_{0.75}$) or maximum voluntary ventilation,[12,19,20,22,29] and increased measures of poor intrapulmonary gas mixing (nitrogen washout

time, lung clearance index).[17,29,51] Increased airway resistance ("interrupter technique") was found in six subjects with Grade II byssinosis.[20] Small, statistically insignificant increases in airway resistance have been found in six subjects with Grade I byssinosis[20] and in nineteen subjects who experienced chest tightness during hemp dust exposure (DuBois' technique; increased resistance expressed as decrease in ratio of airway conductance to thoracic gas volume).[48] In the latter study, statistically significant increases in resistance were measured after dust exposure in six subjects who did not experience chest tightness. Exposure to cotton and hemp dust under laboratory conditions has been associated with chest tightness and with changes of lung function similar to the changes summarized here.[52,53] All the alterations of pulmonary function observed in the acute dust reaction document ventilatory impairment and are quite consistent with the pattern of impairment produced by airway obstruction.[54,55] Obstructive type changes in ventilatory capacity are commonly seen in attacks of bronchial asthma (as in acute dust reactions, these changes are transient and can be measured relative to the individual's usual level of function) and in chronic bronchitis and emphysema (these changes are essentially permanent and are generally measured relative to values expected for an average person of the same sex, age, and height as the individual being studied). The chronic manifestations of byssinosis include evidence of permanent

obstructive ventilatory impairment. When byssinotics such as this, who have initially abnormal lung function values, are exposed to dust, an additional transient obstructive defect may occur.[48] There is some evidence to indicate that this transient obstruction, a manifestation of an acute dust reaction, tends to be of greater magnitude in byssinotics who have reached a chronic stage of disease.[33] Whether the subject has chronic disease or not, during acute dust reactions symptomatic relief and improvement in lung function measurements have been associated with the administration of bronchodilator medications.*

The classification of impairment of ventilatory capacity as obstructive is based on the pattern of external observations and measurements. A number of structural changes which produce airway narrowing (bronchostenosis, bronchoconstriction) could produce the same clinical presentation. However, only a few gross anatomic alterations are plausible as the mechanisms responsible for the transient airway obstruction of the acute dust reaction.[29] There are (1) partial and/or complete occlusion of air passages by bronchial** secretions, (2) thickening of the lining of the

*Adrenaline and atropine compound,[20] isoproterenol,[16,17,30,56] alupent,[57] and isoproterenol-phenylephrine mixture[48] have been used. All were administered by inhalation. Alupent administration prior to dust exposure, by mouth[48] and by inhalation,[57] has been associated with relatively stable $FEV_{1.0}$ and vital capacity measurements.

**Since "bronchial" has several usages,[58] clarification of the definition employed in this paper is necessary. Bronchial has been used as a general term referring to

airways due to edema, and (3) bronchial smooth muscle contraction (bronchospasm). There is no direct evidence that any of these structural changes are present during acute dust reactions. There is direct, post-mortem evidence that each of them occurs and contributes to airway obstruction in bronchial asthma.[59,60]

Elucidation of the cellular-molecular mechanisms which lead to the airway obstruction of the acute dust reaction involves two central questions: what substance or substances in textile dust are active in the production of the obstruction and what is their mode of action.

a. Active substance

Dust from cardrooms of British cotton mills has been examined by light microscopy and microbial culture techniques. The components of the dust identified by various investigators fit into four general categories (after Drummond et al.[61]):

(1) pieces of broken fiber[47,61,62] – known collectively as "fly"; composed chiefly of cellulose; responsible for the major part of the visible cloud of dust; may include fibers up to 1½ inches long.

(2) plant debris[47,61,62] – presumed to derive from leaves, seed husks, pods, and twigs gathered by picking machinery; consists of particles with diameters ranging from about 10 microns to submicroscopic.

---

all the airways of the lungs, including the trachea. Description of a structure or event as bronchial is intended to denote location or occurrence within this system of airways, not necessarily throughout it.

(3) dead and viable bacteria[61-66] – viable soil and water organisms (predominantly species of Bacillus[62,64-66]) have been found in cardroom air in very large numbers; Gram-negative bacilli were not seen microscopically but have been isolated in culture.[62,65]

(4) fungal spores and hyphae[61,62,65,66] – abundant, but less so than bacteria.

The dust also has a mineral content – presumably due to traces of silicate and free silicic acid from cotton plants and larger amounts from soil contamination of raw cotton. These minerals, on the basis of the quantity present and absence of evidence of fibrotic anatomic changes in byssinosis, are not considered pathogenic.[5,28] Dust concentration and the concentrations of the above components of it, seem the same on Mondays as on other days.[28,61] The prevalence of byssinosis in workers has been found to be closely correlated with total dust concentration in their work areas, and more with the protein fraction of that dust than with the cellulose or ash (mineral) content.[28] Attempts to localize the bronchoconstrictor activity of the dust to a particular range of particle size have demonstrated only that the group of particles less than 7 microns in diameter have the activity.[52]

A substance with the biological activity of histamine,*

*β-imidazolylethylamine. The compound was synthesized in 1907 and was isolated from naturally occurring materials in 1910. Early pharmacological study found it a stimulator of smooth muscle contraction and, in larger mammals, a depressor of blood pressure. When histamine was finally indisputably isolated from human tissue in 1927, it was quickly identified with the "H-substance"

was detected in extracts of cardroom cotton dust by Heap et al. in 1932. Macdonald and Maitland, in 1934, found much more of this activity in cotton dust than in coir or esparto grass dust and found it most plentiful in the fraction of cotton dust particles less than 20 microns in length. (Both studies cited by Haworth and Macdonald.[76]) Similar histamine-like actions of dust extract were demonstrated by Prausnitz and Macdonald.[47] Although these two studies estimated different concentrations of histamine-like substance to be present in cotton dust, both figures were fairly small. Chemically pure histamine was isolated from cotton dust by Haworth and Macdonald[76] in 1937. They also found higher histamine levels in the blood of cardroom workers than in the blood of university students or chronic bronchitics. The presence of histamine in dust from a hemp processing factory

---

of Lewis, a substance liberated by cells of the skin following injurious stimuli including the union of antigen and antibody. The characteristic "triple response" of human skin studied by Lewis can be produced either by released endogenous histamine or by histamine pricked or injected into the skin. It consists of (1) localized redness due to dilatation of minute blood vessels (a direct histamine action), (2) a spreading flush, or flare, produced by widespread dilatation of neighboring arterioles (local axon reflex mechanism), and (3) a localized collection of edema fluid, produced by an increased permeability of the walls of fine blood vessels (another direct drug action).[67] Histamine is now known to occur widely in plant and animal tissues.[68] In mammalian tissues, a large amount seems located in the granules of mast cells.[69,70] Histamine, and to some extent mast cells and their granules, have become deeply implicated in the early stages of the response of connective tissue to acute injury, inflammation.[71-75]

was demonstrated using biological methods by Bansagi and Bordas in 1948.[5] Small amounts of 5-hydroxytryptamine* have been found in some cotton dust samples along with histamine and a partially characterized (organic, heat-stable, dialysable, not destroyed by proteolytic enzymes) substance or substances also capable of stimulating smooth muscle contraction.[77-79] One of the cotton dust extracts prepared by Nicholls[78] increased greatly in smooth muscle contracting activity with the appearance of signs of bacterial growth. The activity seemed due to histamine. An experiment was performed to test whether the histamine content of cotton-mill dust could be increased during weekends by bacterial action. Negative results were obtained. Nicholls[78] demonstrated histamine activity and smooth muscle contractor activity in extracts of the pericarp, stem, and bract portions of cotton bolls, indicating a plant origin of these particular active substances in dust extracts. Tuffnell[80] elicited symptoms and a reduction in the maximum breathing capacity in one byssinotic subject with the inhalation of powdered cotton leaf and bract. A similar response was obtained with cotton cardroom dust.

Other experimental studies have indicated the presence

*5-HT, serotonin. Long known as a vasoconstrictor material, the compound was isolated in 1948 and synthesized in 1951. It is widely distributed in plants and animals including man. Its pharmacological actions show great variability with species and organ but generally involve stimulation of smooth muscle and nerve.[67]

of a histamine-liberating substance or substances in textile dusts, originating at least in part from plant material. Bouhuys et al.[81] reported dyspnea and prolongation of nitrogen washout time in two subjects occurring very soon after their inhalation of nebulized cotton dust extract. Neither subject had been previously exposed to cotton-mill dust. The reaction resembled the Monday symptoms of textile workers in that it could not be reproduced the following day but could be elicited several days later. No response occurred when these subjects inhaled house dust extract. These authors also describe a delayed fall in the blood pressure of anesthetized cats following intravenous injection of cotton dust extract. No fall in blood pressure was observed when the extract was injected a second time although the synthetic histamine releaser compound 48/80 produced a second response. A cat pretreated with the antihistamine mepyramine maleate did not respond to injections of either cotton dust extract or compound 48/80. These studies constitute evidence that the cotton dust extract contained a substance capable of releasing endogenous histamine. Antweiler[82] duplicated the effect on cat blood pressure with various cotton cardroom dust extracts and measured increases in the cats' blood histamine content following the extract injections. Antweiler also injected cotton dust extract intraperitoneally in rats and later found rises in the histamine content of their intraperitoneal fluid and less pronounced staining of peritoneal mast cell granules. He observed increases in the

plasma histamine content of heparinized rabbit blood after incubating it with cotton dust extract. The active extracts studied by Antweiler included one produced from material largely composed of shreds of cotton plants and seeds. Nicholls[78] produced blood pressure responses in rats, rabbits, and cats using cotton dust extracts and extracts of pericarp and bracts. These extracts also liberated histamine from rat peritoneal mast cells. Histamine was liberated from the perfused hind quarters of rats by cotton dust and pericarp extracts.

Bouhuys and Lindell[83] obtained evidence of histamine release from pieces of human lung tissue when incubated with cotton dust extract. Nicholls et al.,[84] in similar experiments, demonstrated that extracts of cotton, flax, and hemp dusts and cotton bract extract all were capable of releasing histamine from human lung tissue and not from Guinea pig, rat, cat, sheep, or pig lung tissue. The bract extract was the most active extract studied. Pericarp extract was inactive. Douglas et al.[85] reported similar results; bract extract most active in releasing histamine from human lung tissue. Bouhuys and Nicholls[86] observed obstructive type changes in pulmonary function tests in four subjects who inhaled bract extract. Three of the subjects experienced chest tightness and shortness of breath ten to twenty minutes after inhalation of bract extract aerosol was begun. Repeat inhalations twenty-four hours later were associated with no symptoms and smaller changes of lung function

measurements. A pericarp extract had no effect on two of these subjects.

There is further evidence of the potential of textile dusts and their components for interaction with animal tissue. Some of the biologically active substances already discussed may have had a role in the following studies. Prolonged inhalation of cotton dust[47] and hemp dust[87] by Guinea pigs led to extensive lung tissue reaction. Bronchitis has been described in rabbits following prolonged inhalation of cotton dust extract.[88] Nicholls[78] and Bouhuys and Nicholls[86] found no evidence of an effect on Guinea pigs of short-term exposure to an aerosol of cotton dust extract. Antweiler[89] reported respiratory difficulties in cats and Guinea pigs during exposure to cotton dust extract aerosol. The effects persisted for some time after removal of the animals from the aerosol. Seven of eight Guinea pigs studied died the following day. Antweiler observed no effect in animals exposed to cotton dust.

Intradermal injection of a cotton dust extract in rabbits, Guinea pigs, and one man was associated with a marked local inflammatory reaction characterized by an infiltration of polymorphonuclear leukocytes and some edema.[47] Intravenous injection of cotton dust extract into rabbits is associated with a rise in temperature[77,90] and variations in the numbers of leukocytes in the peripheral blood.[90] Rat plasma anaphylatoxin* was activated by a crude cotton extract.[90] Cotton

dust extracts seemed to cause slow release of a substance which contracted blood-bathed Guinea pig ileum and relaxed blood-bathed rat duodenum.[77] Intravenous injection of dust extracts elicited a slow bronchoconstrictor response in Guinea pigs, slower in onset than the response to histamine or bradykinin.[77] Subcutaneous injections of cotton dust extract in rat paws[82,90] and intracutaneous injection of cotton dust, flax, pericarp, or bract extract in Guinea pigs[78] produced local edema apparently by increasing vascular permeability.

In summary, textile dusts seem to contain copious potentially pathogenic material of plant and microbial origin. That deriving from plants, and, in cotton dust, particularly from the bracts of cotton bolls, has been found to contain small amounts of histamine and 5-hydroxytryptamine and other substances capable of stimulating smooth muscle contraction either directly or through release of endogenous histamine. In many circumstances, brief or prolonged contact between animal tissue and textile dusts in some form has led to tissue damage and manifestations of inflammatory processes.

---

*Anaphylatoxin is a classical designation for a property which can be generated from normal flesh plasma or serum by antigen-antibody complexes and a variety of other agents. Its activities include contraction of smooth muscle, enhancement of vascular permeability, degranulation of mast cells, and release of histamine.[91,92]

b. Mode of Action

The mechanism or mechanisms through which textile dusts produce acute dust reactions in humans are largely a matter of speculation. Particles of any dust less than 10 microns in size are considered capable of penetrating deeply into the respiratory tract[3] and inhaled textile dust particles are presumed to do so. Various hypotheses have been proposed which, in greater or lesser detail, describe biochemical and histologic events subsequent to dust inhalation leading to transient airway obstruction. Most of the hypotheses incorporate explanations for why byssinotic workers tend to experience acute dust reactions only on Mondays. Some suggest that a host factor which is necessary for the development of the reaction may be depleted on Monday and can not be replaced sufficiently for another noticeable reaction to occur until the worker spends at least a day away from the dust. Most of the information already reviewed, concerning components and activities of textile dusts, is suggestive of and/or supportive of one or more of the following modes of dust action.

(1) Toxic. Prausnitz[47] concluded, following his rather extensive animal and human investigations, that the changes which occur in the lungs after prolonged inhalation of cotton dust are the result of a toxic substance or group of substances in the dust. The toxic material seemed to have a "characteristic irritating effect" and to produce "definite inflammatory lesions". Prausnitz suggested that

the prevalence of respiratory attacks on Mondays could be "due to a physical and perhaps a psychical shock occurring when the patients resume their dusty work after one and a-half day's life in a clean atmosphere." He wondered if workers might breath more deeply when they returned from clean air, taking more of the irritating dust into their lungs. The objection has been raised that, if this hypothesis were true, Monday symptoms would occur in most dusty industries. (Interestingly, Prausnitz' discussion mentions a shaleworker with pulmonary fibrosis who reported exacerbations of his symptoms on Mondays.) However, this objection ignores the premise that the dust is especially noxious. Disregarding the role of deep breathing, the non-specificity of this hypothesis makes it a useful general case which includes some of the hypotheses to follow. Its emphasis on irritation and inflammation should serve as a reminder of the gross aspects of mechanisms described on a cellular-molecular level.

Some authors[3,93] have mentioned mechanical irritation with regard to modes of dust action. Given the demonstrated potency of dust to interact with tissue, it is questionable whether a mechanism of the sort implied by the term mechanical could be isolated in any real fashion. In a hypothesis of the pathogenesis of byssinosis which incorporates several active substances and several modes of action, Kovats and Bugyi[5] include factors which allow them to call the disease a toxomycosis. In toxomycoses spores and detritus of fungi

get into the lungs, do not multiply there, but exert a specific effect as foreign bodies.

(2) Microbial. The acute dust reaction has no features suggesting it to be a result of bacterial or fungal infection in any usual sense. However, it is conceivable that dead or viable microorganisms or the products of microorganisms which are present in textile dusts could participate in some way in the pathogenesis of the reaction.[63] There is one extensive hypothesis concerning such a possibility. It overlaps two of the subsequent categories of modes of action, so the microbial category has been introduced to emphasize its most essential element.

It has been suggested that Monday reactions are produced, in whole or in part, by endotoxins of Gram-negative bacteria which contaminate textile dusts. Similarities have been noted between effects of inhaled vegetable dusts and certain effects caused by endotoxins. Evidence of endotoxin-like action of cotton dust and its extracts has been accummulated,[90] and biological assay of endotoxin-like substances in cotton dust has yielded high concentrations.[94] Following the demonstration by Bouhuys et al.[81] and Antweiler[82] of histamine releasing activity in cotton dust extracts, this activity, or part of it, was attributed to endotoxins by those who had proposed the endotoxin hypothesis.[90,95] Antweiler[96] objected to this and reported that homogenates of organisms (Bacillus subtilis, B. megaterium, B. cereum, and Aspergillus niger and A. flavus) cultured from cotton dusts used in his original

animal experiments had been tested and had not released histamine. He then presented new evidence that pure endotoxins, in amounts such as might be present in cotton-mill dust, were not capable of releasing histamine. He also pointed out that one of the cotton dust extract activities attributed to endotoxins is stabile to boiling in acid while endotoxins are not.

Endotoxins are characteristically pyrogenic and there has been general acknowledgment that they may have a role in the pathogenesis of mill fever. There has been little acceptance of the hypothesis that they are important in byssinosis, which is not associated with fever.[8] In part to answer this objection to their endotoxin hypothesis, Cavagna et al.[97] proposed an immunologic mechanism of byssinosis. They suggested that the concentration of symptoms early in the week could be due to the host passing through cycles of hypersensitivity and tolerance. Using high doses of endotoxin and dust extract they produced intense inflammatory bronchial reactions in sensitized rabbits and obtained some evidence of crossreactivity between the extracts. Skin tests of byssinotics reported by Heaphy and Kilburn[98] indicate that allergy to microbial material may have a role in byssinosis, while serologic, skin, and inhalation tests reported by Wuthrich[99] do not.

(3) Direct pharmacological. The histamine, 5-hydroxytryptamine, and smooth muscle contractor(s) in textile dust, if they have pathogenetic significance, would presumably

act pharmacologically on lung tissue. They might produce bronchospasm by affecting the membranes or ionic environment either of bronchial smooth muscle cells or of the nerves which supply them. Histamine, at least, might promote edema formation by increasing the permeability of small vessels in the airway lining. Not enough is known about the concentrations of pharmacologically active substances in dusts, or about local factors which would influence their absorption and effectiveness, to make a definite statement about their role in the acute dust reaction. There are some facts which have been taken as evidence against the importance of histamine and 5-HT. The concentrations of histamine in inhaled aerosols necessary to rapidly induce lung function changes in byssinotics[100] seems to far exceed the reported concentrations of histamine in textile dusts.[47] (Lung function changes in the acute dust reaction develop gradually.[20]) The presence of 5-HT in samples of textile dusts has been an inconstant finding.

The effect of antihistamine drugs on the acute dust reaction has implications for hypotheses of the pathogenesis of that reaction which include roles for either exogenous (the case here) or endogenous (the two cases to follow) histamine. Antihistamines antagonize the constrictor action of histamine on respiratory smooth muscle in man and effectively antagonize the action of histamine that results in increased capillary permeability and the formation of edema.[67] They tend not to protect man against allergic bronchospasm,

possibly because histamine is not the only pharmacologically active agent mediating that reaction and/or because endogenous histamine is released in such intimate association with bronchial smooth muscle that there is little possibility of its inhibition. The capability of antihistamines to influence acute dust reactions seemed at first equivocal,[15,17,20] but in controlled studies two of these drugs (methdilazine hydrochloride[34,101] and N-benzhydryl-N'cinnamyl-piperazine[93]) have tended to prevent acute dust reactions.

(4) Allergic. Inflammatory processes initiated by reactions between allergens and humoral or cellular body constituents specifically altered to engage those allergens are termed allergic. They are thought to be mediated by pharmacologically active substances released from storage sites and/or changed from an inactive to an active form as a result of the initial reaction. Mediators identified in some mammaliar tissues include histamine, slow reacting substance,* 5-hydroxytryptamine, plasma kinins,** and various "permeability factors"[105]. Such mediators, especially histamine, are considered capable, though not necessarily individually,

*Material or materials which contract smooth muscle more slowly than histamine or acetylcholine and are not antagonized by mepyramine.[102,103]

**Denotes the kallidins and related polypeptides. Kallidin I or bradykinin has demonstrated five pharmacological activities: smooth muscle stimulation, vasodilatation, increase in capillary permeability, migration of leukocytes, and stimulation of pain fibers.[102] Brocklehurst[104] discusses the distribution and activation of the plasma kinins and their possible significance in human allergies.

of producing manifestations of airway obstruction in man. An allergic inflammatory reaction is considered the basis of the pathogenesis of bronchial asthma.[60] The bronchospasm, edema, and perhaps even the increased bronchial secretion in that disease, may be effects of these mediators.

In 1937, when few details of allergic mechanisms were known, Haworth and Macdonald[76] speculated that the acute dust reaction in byssinosis might be the result of the combined action of histamine in cotton dust and allergically released endogenous histamine. They suggested that an accumulation of histamine in the lungs of workers over the weekend might account for the severity of reactions on Monday. Today there is much more information, factual and theoretical, available concerning mechanisms in allergy. Allergic reactions which produce tissue damage and are known to operate in clinical hypersensitivity have been classified according to four basic mechanisms.[106] Of the four mechanisms, two are believed to have relevance in human reactions to inhaled organic antigens.[107] Histamine has been studied as a mediator of both types of reaction.

Type I (immediate, anaphylactic) hypersensitivity is generally thought of as occurring in human subjects with an allergic diathesis who are termed atopic. These individuals seem to spontaneously develop, under hereditary influence, allergic diseases such as asthma or hay fever. The basis of their disease-producing immunologic activity seems to be antibodies with skin-sensitizing (homocytotropic) properties

called reagins. The functions of reaginic antibody seem to be performed largely, though perhaps not exclusively, by immunoglobulins of the new class IgE (IgND).[107-109] There is normally some IgE in human serum but atopic individuals evidently have elevated levels.[110-112] Skin-sensitizing antibodies can be induced in normal and atopic subjects, as in penicillin or insect bite hypersensitivity, but it is not yet clear that these antibodies are the same as the reagins of atopics[107,113] or that the disease manifestations they produce develop by a Type I mechanism. In Type I reactions reagin is first produced and then becomes bound, by processes which are not understood but which require time, to cells which contain mediators of inflammation (such as mast cells, blood basophils, polymorphonuclar leukocytes, possibly some vascular endothelial cells). Union of antigen with cell-bound antibody is believed to activate a system responsible for release of mediators from the cell. Activation of this system requires calcium and seems to involve a heat-labile component of tissues. The entire process of release requires energy and can be chemically inhibited.[105,106,114] Although evidence on the point has been equivocal in the past,[115] there is now strong evidence that complement is not involved in this release mechanism,[116] or at all in reaginic hypersensitivity reactions.[109] Experimental preparations have demonstrated that Type I hypersensitivity can become refractory to further stimulation by antigen and, to return to speculation about the limitation of the symptoms of byssinosis to

Mondays, the refractoriness may be due to depletion of antibody, mediator substances, or any of the factors of the system involved in mediator release.

Type III (Arthus-type, damage by toxic complexes) hypersensitivity elaborates on the observation that complexes between antigen and antibody formed in moderate antigen excess are locally toxic to tissues. Such complexes can be formed when antigen is circulating in the blood and antibody is beginning to be produced ("serum-sickness" situation) or when the antibody is circulating in the blood and antigen is introduced into the tissues in high concentration (Arthus-type situation). Formation of these complexes is followed by local histamine release; attraction of circulating leukocytes; an accumulation of leukocytes, platelets, and fibrin which may lead to thrombosis and hemorrhage; and, due to systemic absorption of chemotactic factors, agglutination of leukocytes distant from the complexes.[106] All these inflammatory changes are apparently wholly complement dependent.[117] It has been known for some time that elements of the serum complement (C') system become bound to some antigen-antibody complexes. However, it has only recently been appreciated that, with the activation of the complement system produced by antigen-antibody complexes, anaphylatoxins are produced. Low molecular weight fragments split from the third component (C'3) and from the fifth component (C'5) of the complement system promote enhanced vascular permeability, smooth muscle contraction, and chemotaxis. Histamine is released and may

produce some of these events. (Interestingly, the C'3 and C'5 anaphylatoxins appear to release histamine from separate tissue stores,[118] a property which seems to be shared by some exogenous histamine releasing compounds.[119] The apparent limitation of the amount of histamine which can be liberated by one histamine releaser constitutes evidence against the suggestion that total lung histamine is depleted on Mondays in byssinosis.) Since Type I and Type III allergic reactions have some final mediators in common, their histological and clinical manifestations may be quite similar. The reactions seem to stem, however, from different types of antibody. In experimental models of Type III reactions, no time interval is required before antigen can evoke a reaction from antibody-injected tissues, indicating that binding of antibody by cells is unnecessary. Once initiated, the manifestations of Type III reactions develop more slowly than those of Type I reactions. The intensity of Type III reactions may be proportional to the level of circulating precipitating antibody specific for the allergen involved. Precipitating antibodies are considered the basis for Type III reactions in the same way that reagins, and possibly the skin-sensitizing antibodies of non-atopics, are considered the basis for Type I reactions. Tolerance to an allergen which produces tissue damage by a Type III mechanism could presumably be induced by depletion of antibody, complement, mediators of inflammation, or unidentified factors involved in the release of mediators by anaphylatoxins. Pepys,[107] who is

largely responsible for the recognition of the importance of Type III allergy in reactions to inhaled organic antigens, points out that the Type I and Type III categories are not necessarily discrete. A single subject may show both Type I and Type III hypersensitivity to the same allergen, with one type of reaction tending to dominate the other in the production of disease.

In general, to establish that particular disease manifestations have an allergic pathogenesis by a Type I or Type III mechanism (adapted from Pepys[107]), antibody to the suspect allergen must first be demonstrated. If antibody is present, there is evidence only of exposure to the allergen or to related allergens. The antibody need not have pathogenetic importance. To be implicated in disease, the antibody must be capable of mediating allergic reactions and should be correlated with, and relevant to, the clinical disorder under investigation. There is evidence that antibodies to textile dust antigens are present in both textile workers and non-textile workers. As the following review of this evidence indicates, none of the antibodies detected have fulfilled all these criteria of pathogenetic importance.

Specific reagins or other skin-sensitizing antibodies can be both detected and shown to be capable of initiating a Type I hypersensitivity reaction by skin tests. Evidence that a subject has skin-sensitizing antibody to a specific allergen is obtained if an urticarial wheal and an erythematous flare, maximal in ten to twenty minutes, develops at

the site where the allergen has been pricked, scratched, or injected into the skin. The skin changes themselves are a hypersensitivity reaction and constitute a positive immediate, Type I skin test in humans.[120] Cayton et al.[121] obtained positive immediate skin reactions to extracts of cotton dust in several byssinotic and non-byssinotic cotton workers and in allergic and normal controls. The positive tests in workers did not correlate with the presence of disease. The possibility that the positive tests in controls were due to sensitization by a widespread antigen was supported by the finding of similarities in chemical and skin testing properties between house dust and cotton dust extracts.[122] Most other studies of immediate-type skin hypersensitivity to textile dusts have found a similar lack of correlation between positive skin tests and clinical byssinosis with negative tests occurring frequently in byssinotic workers, positive tests in non-byssinotic workers, and positive tests in unexposed controls.[26,40,65,99] Prausnitz[47] has found positive skin reactions, probably of the immediate type, in all the diseased cotton operatives he tested and in few controls. He obtained additional evidence that the reactions were produced by skin-sensitizing antibody when four of five sera from byssinotic workers gave positive Prausnitz-Kustner tests. (The studies cited vary with respect to materials selected for extract preparation and extraction procedures.) Inhalation tests with textile allergen preparations have been positive in relatively small proportions of the byssinotic

workers tested.[26,40] Byssinotics do not show the extreme sensitivity to inhaled histamine that is characteristic of atopic patients with bronchial asthma.[123,124] If textile dusts have a single mode of action in producing byssinosis, most of these results constitute evidence that it is not a Type I hypersensitivity mechanism.

Reading skin tests to textile dust extracts is considered difficult because non-specific irritative reactions may be taken as positive.[125] Prausnitz[47] and Cayton et al.[121,122] observed delayed reactions to cotton dust extracts which they considered due to toxic factors. It is worth noting, however, that some of the characteristics of the reactions they described resemble those of Arthus type reactions.[107,120] The association of some of these reactions with Type I skin responses, their time course (maximal at 10-12 hours[121]), and the extent of tissue involvement occasionally observed all suggest that they could be Type III reactions resulting from precipitating antibody. Their occurance in nearly all subjects, both textile workers and controls[121] would have to be explained. Hypotheses of natural antibody or ubiquitous antigen could perhaps be evoked. The house dust extract of Cayton et al.[122] did not elicit delayed reactions.

Lopez Merino et al.[126] described delayed skin responses to hemp dust extracts, maximal in thirty-six hours, which resembled the cutaneous reaction of Type IV or tuberculin-type hypersensitivity. Similar reactions, to several textile allergen preparations, were observed by Popa et al.[26] At

present, however, there is no reason to consider Type IV hypersensitivity relevant to any of the manifestations of byssinosis.

When present, precipitating antibody, unlike reagins, circulates in the blood in concentrations which often permit its detection by various serologic techniques. In 1964, Massoud and Taylor[42] reported that they were unable to demonstrate antibody with several of these techniques using various crude and refined antigen preparations. However, by gel double diffusion they obtained positive precipitin reactions with all human sera tested, which included specimens from cotton cardroom workers and non-textile workers, to a crude saline extract of the receptacle of the cotton flower (evidently distinct from the bracts and pericarp since the authors mention other antigen preparations made up from these cotton plant parts). Taylor et al,[43] subsequently reported isolation of a purified antigen (4' tetrahydroxyflavone-34 diol) which, in a double blind inhalation study,[127] produced symptoms of acute dust reactions without changes in lung function in five of six byssinotic cotton workers but not in non-byssinotic cotton workers or in controls previously unexposed to the cotton industry. The precipitating antibodies found in Massoud and Taylor's early work promoted the hypothesis that the acute dust reaction results from an Arthus Type III allergic reaction.[107] Wuthrich[99] found precipitating antibodies to cotton dust extract in the sera of three of the four cases of byssinosis which he studied, also using the

gel double diffusion method. He also obtained positive inhalation tests in all five cotton workers studied (four diagnosed as byssinotic, one as allergic bronchial asthma due to cotton dust), but not in control subjects. The allergic hypothesis of acute dust reaction pathogenesis seems to now rest on less evidence. At a meeting on byssinosis in the spring of 1970, Taylor reported that the precipitin reactions described in the 1964 paper were apparently due to tannins in the antigen preparation which reacted non-specifically with serum.[128] The concentration and purity of antigen are important in double diffusion tests and another type of false positive reaction has reportedly occurred frequently in double diffusion testing for allergy to other inhaled organic dusts.[129] Using allergens prepared by standard methods as well as crude extracts (all from material apparently consisting largely of textile fibers), Popa et al.[26] were unable to demonstrate precipitating antibodies by gel double diffusion tests of sera from byssinotic and non-byssinotic textile workers or from unexposed controls. They did, however, obtain evidence of circulating antibodies to all their antigens in all sera tested by the tanned red cell agglutination method. The agglutinating antibodies were not further characterized.

(5) Indirect pharmacological – histamine liberation. Any chemical that causes frank tissue damage will liberate endogenous histamine. Some substances, however, can release histamine with minimal evidence of injury, exerting their

action chiefly on mast cells. Compounds with this property include proteolytic enzymes, such as trypsin; surface active materials, including detergents, bile salts, and lysolecithin; a group of high molecular weight substances which includes dextran and ovomucoid; many organic bases, including numerous drugs in common clinical use (stilbamidine, d-tubocurarine, morphine, various antihistaminics); and synthetic substances, compound 48/80 and compound 19/35L, with exceptionally potent histamine-liberating power.[67] MacIntosh and Paton[130] list sixty-four organic bases with which they obtained delayed depressor effects on cat blood pressure of the type characteristically produced by histamine liberators. Selye[75] emphasizes that "any compound – including even distilled water or physiologic salt solutions – will cause topical mast cell degranulation if injected in sufficiently large amounts. The ability of certain compounds to produce mast cell degranulation specifically (at low dose levels and with the least possible side effects) is essentially a matter of degree." He then presents (pp. 148-217) a long alphabetical list of drugs whose effects on mast cells have been examined and includes short abstracts of papers describing those effects. The mechanism of mast cell discharge due to these pharmacological agents seems to vary. Some agents can act directly on the mast cell to produce degranulation and a release of histamine, while others act indirectly by damaging adjacent tissues or by splitting humoral substrates and releasing substances which are then toxic to the mast cell.[131]

Given the common occurence of the histamine releasing property, it is not surprizing that such activity has been demonstrated by textile dust extracts. It remains to be determined whether that activity is incidental to byssinosis or whether it is important in the causation of the disease. The latter possibility has interested a number of investigators, perhaps in part due to the biologic plausibility of the hypothesis that the intense dustiness of a few industries could provide a relatively unique opportunity for a common property of environmental material to produce disease. Statements of this hypothesis employ the pharmacological term tachyphylaxis to refer to the apparent inability of histamine releasers in dust to act throughout the week. The basis for the tachyphylaxis could be depletion of the histamine releasing stores available to the histamine releasing compound(s) or depletion of factors involved in the release of histamine by those compound(s).

2. Chronic lung disease

Late in the natural history of byssinosis, affected textile workers and ex-workers present the same disease

manifestations as people debilitated by chronic bronchitis and emphysema. The nature of these disease manifestations is suggested by the following generally accepted definitions:

> Chronic bronchitis is a clinical disorder characterized by excessive mucous secretion in the bronchial tree. It is manifested by chronic or recurrent productive cough. Arbitrarily, these manifestations should be present on most days for a minimum of three months in the year and for not less than two successive years. Many diseases of the lung, e.g., tuberculosis, abscess, and of the bronchial tree, e.g., tumors, bronchiectasis, as well as certain cardiac diseases, may cause identical symptoms; furthermore, patients with chronic bronchitis may have other pulmonary or cardiac diseases as well. Thus, the diagnosis of chronic bronchitis can be made only by excluding these other bronchopulmonary or cardiac disorders as the sole cause for symptoms.[132]

> Emphysema is an anatomic alteration of the lung characterized by an abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchiole, accompanied by destructive changes of the alveolar walls.[132,*]

(Emphysema has been given this morphological definition because the structural alterations may exist without clinical manifestations and because the clinical manifestations which may be present are not unique to emphysematous lesions. However, those manifestations – dyspnea, cough, wheezing,

*A somewhat more inclusive definition of emphysema, with subclassifications, is now usually employed pathologically.[133] The definition quoted above, however, is more closely related to the "emphysema" which has been studied epidemiologically and clinically and to which this paper must make considerable reference. The assumptions expressed in the text of this paper concerning the etiology of emphysema as therein defined do not violate what is known about the etiology of the relevant subtypes (panlobular and centrilobular) of the pathologist's "emphysema".

prolongation of expiration, overexpanded thorax, hyperresonance to percussion, x-ray signs of pulmonary overinflation, obstructive ventilatory changes – would be anticipated to result from the anatomical lesion.)

Criteria for the presence of either chronic bronchitis or emphysema and most often for both, are met by a large number of individuals afflicted with what may be considered an end-stage lung disease. These people demonstrate obstructive ventilatory impairment and degrees of respiratory insufficiency. Beyond these physiological derangements and the usual presence of excess mucus and emphysematous alterations of lung structure, there is little which characterizes the entire group. As patients, members of the group present similar problems and essentially one set of therapeutic measures is used in their medical management. It has therefore been found convenient to regard them as having a single disease entity, "chronic obstructive lung disease" or "chronic nonspecific lung disease". It is difficult to specify the etiology of chronic obstructive lung disease because individuals seem to reach what constitutes a final common pathway of disease by different routes. (Hence the designation "end-stage".) A concept of etiology can be derived, however, by considering the disease to result from the interaction over time of numerous host and environmental factors in individual combinations. In the combination of factors contributing to the development of chronic obstructive lung disease in one person, only a portion of all the factors capable of contrib-

uting to the development of the disease might be present. Further, the relative importance of the factors might vary considerably from individual to individual. Host factors found to be statistically associated with chronic obstructive lung disease and which seem, from biological considerations, to have etiologic importance, include advancing age,[134] male sex,[134] and the presence of bronchial asthma,[60] mucoviscidosis,[135] or α_1-antitrypsin deficiency.[136] Environmental factors with similar significance include climate,[137] tobacco smoke,[138] atmospheric pollution,[138-140] and occupational exposures.[137,142] While there conceivably could be a final common pathway of disease mechanism (inflammation resulting from bacterial infection has received considerable attention in this regard[133]) as well as of pathophysiology and morbid anatomy, it would appear from the diversity of the factors already implicated in the etiology of chronic obstructive lung disease that its pathogenesis involves several mechanisms. Chains of molecular-cellular events seem to begin with items as diverse as congenital genetic abnormalities and inorganic inhalents.

Byssinotics with advanced disease fall within the group meeting the criteria for chronic obstructive lung disease. Clinical evidence, including radiographic findings,[47] supports the application of this category as does the small amount of pathologic evidence which is available. This pathologic data is briefly reviewed in the following paragraph.

Prausnitz[47] summarized the findings of Dunn and Sheehan in autopsies of ten cases of byssinosis. In nine cases they

found "definite changes of the respiratory system... of the type of bronchitis and chronic emphysema." Right heart dilatation was a frequent related finding. Schilling[2] cited Dunn and Sheehan's report and a few other descriptions of similar findings. He also quoted a communication from Gough describing changes in the lungs of three deceased cotton workers said to have had byssinosis. Gough noted, in addition to generalized emphysema and chronic bronchial inflammation, the presence of black foci of dust surrounded by tissue reaction. These "dust bodies" were also reported by De Boer[143] as occurring in the emphysematous lungs of a man who had worked with cotton waste for many years. Apparently byssinosis was a post mortem diagnosis in Gough's case, though the case history records a chronic productive cough dating from the time of cotton waste exposure. (Byssinosis was demonstrated in the cotton waste industry in 1950, but publication of the study was relatively recent.[144]) De Boer also found mucopurulent bronchitis with mononuclear and plasma cell infiltration and areas of massive fibrosis. Antemortem, an elevated serum gamma globulin level had been found. Whether or not dust bodies should prove to be a specific lesion in byssinosis, the anatomic alterations of chronic obstructive lung disease which have been consistently found in byssinotics would be expected to have greater pathophysiological significance.

Endoscopy and biopsy are invasive clinical techniques which sometimes bridge the hiatus between clinical and pathological studies. The application of such techniques to the

study of byssinosis has been limited and, as with the pathology of the disease, firm conclusions must await further study. Merlo and Monteverde[145] bronchoscoped a number of workers with occupational respiratory diseases. In twenty cotton workers, each of whom had over twenty years exposure to dust and manifested severe chronic lung disease, they observed bronchial mucosae which were congested and showed evidence of brisk tone. Similar changes were noted in one hemp worker with a history of acute dust reactions. (In this man, a small polyp was demonstrated in a segmental bronchus of the right lower lobe. It was presumably an incidental finding, but it provides an interesting example of a type of gross anatomical change not among those considered to be plausible as causes of the manifestations of byssinosis but which could produce obstructive type changes in airway function.)

It seems, then, that exposure to textile dust is among the environmental factors which may contribute to the development of chronic obstructive lung disease. It has been recognized that tobacco smoke, atmospheric pollutants outside the place of work, and respiratory tract infections should all be taken into account when evaluating the pathogenesis of lung disease in dust-exposed textile workers.[8] There is recent evidence, obtained retrospectively, that textile dust exposure promotes the development of chronic lung disease to a greater extent than does cigarette smoking.[25,146]

The mechanism or mechanisms through which textile dust

promotes the anatomical and physiological changes of chronic obstructive lung disease are unknown. Some of the mechanisms proposed for the acute dust reaction could be involved. Some proponents of unitary hypotheses of the pathogenesis of the acute dust reaction have speculated that one mechanism could lead to both the acute and chronic manifestations of byssinosis. They have wondered if permanent lung damage could accrue from the numerous acute dust reactions experienced by byssinotic workers with years of dust exposure. To examine the relative importance of all the factors which could contribute to the development of chronic obstructive lung disease in textile workers, including acute dust reactions, well controlled prospective studies would be necessary. We might hope that programs of primary and secondary prevention will soon make such studies impossible.

Earlier in this paper emphasis was placed on the role of the acute dust reaction in making an early diagnosis of byssinosis for purposes of the secondary prevention of disabling lung disease. It is important to realize that present evidence does not sustain the conclusion that acute dust reactions are necessary in the pathogenesis of the permanent lung damage associated with years of textile dust inhalation. This means that a secondary prevention program employing diagnostic criteria sensitive only to acute manifestations of byssinosis could fail to protect some individuals from chronic lung disease. The functional grading system[44] proposed for use in secondary prevention programs circumvents

this problem by measuring both reactivity to dust and respiratory impairment present when there has been no recent dust exposure.

As Theodore et al.[147] have pointed out, pulmonary physiology, pulmonary pathology, and epidemiology have provided information about chronic obstructive lung disease without particularly elucidating the cellular-molecular mechanisms involved in its pathogenesis. However, with regard to the role of the acute dust reaction in the development of chronic lung disease in byssinosis, it is interesting to note that there may be correspondence between the site of airway obstruction in acute dust reactions with that in chronic obstructive lung disease. Evidence has been obtained, from tissue studies employing a combination of physiological and anatomical techniques, which indicates that the site of airway obstruction in some patients within the spectrum of chronic obstructive lung disease is in peripheral airways (those airways less than 2 mm. in diameter) and that mucus seems the most important gross cause of obstruction.[148,149] Pulmonary physiologists who have studied lung function changes during acute dust reactions have had the impression for some time that the functionally important dust effect is on "small airways".[52,86] Refinements in respiratory mechanics theory[150] and further study of the acute dust reaction[48,49] have tentatively defined these small airways as those which are not compressed during forced expirations. The peripheral airways designated by this physiological definition may or may not correspond

exactly with those in the anatomical studies. The point of interest at present is simply that there seems to be some correspondence between the two. Tobacco smoke has also been shown to affect airway dynamics.[151-155] Nadel and Comroe[151] indicate that the effect is on peripheral airways. Bates[156] has constructed a hypothesis of the pathogenesis of chronic obstructive lung disease which emphasizes somewhat different effects of tobacco smoke on small airways.

As mentioned earlier, the plausible gross causes of obstruction to (peripheral) airways during the acute dust reaction are bronchial secretions, swelling of the bronchial lining, and bronchospasm. Since sputum production is not remarkable in the early stages of byssinosis, mucus has been assumed to contribute very little to obstruction at that time. However, at a later stage of the disease, when the excessive secretions of chronic bronchitis are present, it seems likely that mucus becomes important. Descriptions in the early literature of acute dust reactions in chronically diseased workers indicate that the reactions were quite dramatic. Evidence indicating that the acute changes in lung function of chronically diseased workers are larger than those of other workers has already been cited (P.18). Massoud et al.[124] observed that cotton workers with chronic bronchitis developed much larger obstructive ventilatory defects in response to inhaled histamine than did workers without chronic bronchitis. In each of these situations, mucus in the airways of the byssinotics may have been an important contributor to

the magnitude of the changes observed. A hypothetical experiment illustrates the principle which may be involved. Assume two identical lengths of airway have been prepared so that the resistance to air flow through them can be measured and that one of them is partially obstructed by secretions. (The initial resistance of the partially obstructed airway would of course be somewhat higher than that of the unobstructed one, but this does not interfere with the comparison of changes to be made.) If a given, equal amount of bronchospasm or swelling of the bronchial lining could then be induced in both lengths of airway and the resistance through each remeasured, the change in the resistance of the partially obstructed airway would be expected to be considerably greater than the change in the resistance of the unobstructed one. The experiment has been defined so that the internal diameter of the partially obstructed airway is initially smaller than that of the unobstructed one and that both internal diameters are then reduced by the same amount. The expectation for changes in resistance assumes that the flow of air approximates laminar flow. The result reflects the well known property of fluids expressed in Poiseuille's law.*

*Although these comments suggest that the basic gross mechanism of acute dust reactions involves only swelling and/or bronchospasm, it is not inconceivable that secretions could have a significant role. The writer has refrained from discounting any of the plausible contributors to airway obstruction in the acute dust reaction. Some authors have been less reluctant to draw conclusions from the information at hand. They not only consider

3. Summary and comment on pathogenesis

Several active substances which may have a role in the pathogenesis of the acute dust reaction have been identified. There has been considerable speculation about the mode of action by which these or other substances elicit the acute dust reaction and contribute to the development of chronic

---

the effect of secretions negligible because of their absence early in the course of the disease (Often no mention is made of their presence and possible significance later in the course of the disease.), but they also tend to minimize the role of swelling on the basis of the response of the acute dust reaction to bronchodilator drugs. When they relieve airway obstruction these medications are believed to act predominantly on bronchial smooth muscle. However, some of the bronchodilator ingredients (adrenaline, phenylephrine) would be expected to have a decongestant effect on bronchial mucosa. Such a vasomotor (local vasoconstrictive, α-receptor stimulative) effect is not generally expected of isoproterenol, which has a characteristic stimulative effect on β-receptors and is a potent relaxer of human bronchial smooth muscle. However, there does not seem to be sufficient evidence to conclude that inhaled isoproterenol does not have a constrictive effect on small vessels of the bronchial lining. Given intravenously in man, isoproterenol lowers peripheral vascular resistance mainly by its effect on the vasculature of skeletal muscle, with some contribution from the renal and mesenteric vascular beds. In conventional doses neither a rise nor a fall in pulmonary blood pressure has been found in man. In addition to this β-receptor stimulation, isoproterenol has shown some capacity to stimulate α-receptors. An α-receptor action on some muscles is seen when the drug is administered in high doses, and when injected intradermally in man it causes blanching of the skin.[157] (The recent textbook review by Innes and Nickerson[157] is the writer's source for the pharmacological information included in this footnote. It was not contradicted by papers on isoproterenol in the pharmacological literature which were also consulted. The controversy implied here is not a new one and has received considerable attention with relation to asthma.[158])

lung disease. Investigators have tended to favor unitary hypotheses which implicate only one substance and one mode of action. One of the factors which has contributed strongly to the preference for unitary hypotheses -- the desire to render byssinosis completely preventable -- is the most basic reason for interest in the pathogenesis of byssinosis. There is evidence that workers exposed to dust produced from textile raw materials which have undergone nonspecific early treatments experience much less respiratory difficulty than workers elsewhere in the industry.[14,159,160] Should a unitary hypothesis prove correct, a simple and inexpensive means of specifically deactivating dust might be devised. An additional reason, which is separable from preventive concerns, for interest in the pathogenesis of byssinosis has emerged as the study of pathogenesis has advanced. It has become evident that elucidation of the mechanisms of the acute dust reaction and of the chronic lung disease of byssinosis should produce information of fundamental biologic significance. Airway reactivity is among the general topics which have already been examined in relation to byssinosis, ageing and the lung is among those which deserve detailed consideration in the future.

Serologic Study: Rationale and Goals

Two serologic studies, with implications for the allergic and indirect pharmacological (histamine liberation) hypotheses of the pathogenesis of the acute dust reaction of byssinosis, have been carried out. The following pages provide background material on the techniques used, examine reasons for considering the information obtained important, and discuss factors which must be taken into account in drawing conclusions from the work.

1. Serum complement

The complement system is known to be an important mediator of host reactions to foreign substances which, by a number of possible routes, gain entrance to host tissue. Some of the reactions have protective value while others, with or without having a role in host defense, constitute manifestations of disease. Reactions involving complement which have received considerable attantion are immune cytotoxicity and acute inflammatory responses. Among the mechanisms proposed for the pathogenesis of the acute dust reaction, at least two could be mediated by complement.

a. Technical considerations

The complement (C') system has been identified and characterized mainly through its mediation of immune cytotoxicity. The eleven distinct serum proteins which comprise the nine functional components (C'1-C'9)* of the complement

*Of the several available reviews on complement, the more

system all take part, some as enzymes, in the cascade of reactions which leads to the immune lysis of erythrocytes. Until relatively recently, assay of complement was dependent upon this hemolytic activity. Techniques for measuring complement levels in terms of hemolytic activity are precise, but are cumbersome. Many of the difficulties involved in performing these measurements are suggested by the definition of the unit in which the levels are usually expressed: "The hemolytic unit of complement, $C'H_{50}$, is defined as that amount in milliliters which will lyse 2.5×10^8 optimally sensitized red cells out of a total of 5×10^8 cells, in the presence of optimal Ca^{++} and Mg^{++}, at an ionic strength of 0.147, in one hour's incubation at 37 degrees C, in a total volume of 7.5 ml."[167] To assay a particular specimen, serial dilutions must be tested. The level measured in a given specimen reflects the activity of all the components of the C' system; it does not provide quantitative information concerning individual components.

There are abnormalities involving the C' system which affect only single components. The mediators of the acute inflammatory response which derive from components of the C' system can be produced by interaction of fewer than all nine components. For these and other reasons, the components of

---

recent articles[92,117,161] use the nomenclature assumed above for the components of complement. These designations have had general acceptance since 1966.[162] The reviews cited, together with earlier publications,[163-166] indicate the terminological adjustments which have been necessary as information about the C' system has increased.

complement are thought to have different biological roles and in assessing the role of complement in disease it is considered important to analyze them individually.[168] By preparing cells which are in intermediate stages of the sequence which leads to hemolysis, relatively quantitative measurements of all nine components of complement have been performed. These tests, however, are even more laborious than the $C'H_{50}$ (50% hemolysis) method of measuring whole complement. Fortunately, some components of the C' system have been isolated biochemically, and methods of studying them have been developed which are not dependent upon their hemolytic activity.

Muller-Eberhard et al,[169] isolated a β_{1C}-globulin related to the C' system.[170] Further study, and reclassification of C' components, rendered β_{1C}-globulin identical with the third component (C'3) of complement,[171] This protein, which is present in relatively large amounts in normal human serum,[172] undergoes gradual spontaneous conversion in vitro to a lighter, hemolytically inactive protein (β_{1A}-globulin) by a process which is complete within six days at 37 degrees C, and four to six weeks at 1 degree C,[169] Although the β_{1C}- and β_{1A}-globulins have different physical properties, their antigenic structure is similar and specific antibodies engendered in animals react with both molecules. Antisera to these proteins have been used to measure serum C'3 levels by immunoassay techniques (the tests can be performed even after protracted serum storage[173]) and to localize β_{1C}-globulin

in tissues by fluorescence microscopy. Immunoassay of fresh human serum β_{1C}-globulin has shown that it must fall below about 60% of its normal value before a decrease in hemolytic serum complement becomes evident.[174] Immunoassay methods, which are far more convenient than the hemolytic techniques, have also been used to measure serum levels of C'4, C'5, and one of the three components of C'1.[168]

Single radial immunodiffusion has been used in several studies to measure immunoglobulin and β_{1C}/β_{1A}-globulin serum levels. It became popular as an assay technique at about the same time that antibody to human C'3 became widely available. The method depends upon a precipitin reaction which is produced by first incorporating at a uniform concentration, one of a pair of immunological reactants (usually antibody) into a layer of agar gel. The other reactant is then allowed to diffuse into the gel from a well cut in the gel. A disc or halo of immune precipitate gradually forms around the well. Its size provides a measure of concentration. The term single is applied to the technique to distinguish it from double immunodiffusion, in which neither reactant is in the gel initially and both diffuse from wells. Radial indicates the direction of diffusion of the second reactant from the well and distinguishes the technique from single immunodiffusion in tubes, where the second reactant is placed on top of the gel containing the first reactant and then diffuses linearly. The phenomenon of single radial immunodiffusion was evidently discovered by accident by Petrie[175] in 1932. Ouchterlony[176]

put the reaction to semi-quantitative use in 1948, but there was little use or refinement of the technique for some time. Mancini et al.[177] acknowledge techniques described by other authors between 1957 and 1963, but it seems to be their approach which has had the greatest influence on the commercial tests now in wide use.[178]

The following theoretical molecular description of a radial immunodiffusion test should facilitate understanding the method used to measure C'3 levels in this study. It is assumed[179] that the kinetics of precipitin formation in single radial diffusion are quite similar to those[180] which seem to occur[181] when diffusion is linear. Consider the test to have been prepared with specific antibody in the agar and that material containing the antigen is introduced into a well in the gel layer. Antigen rapidly enters the gel by a process involving both diffusion and capillary attraction. In the neighborhood of the well it is in a much higher local concentration than the antibody already in the agar. Some of the antigen combines with the available antibody to form precipitin, but the antigen is in such excess that it continues to diffuse. As it spreads outward it becomes less concentrated. When it encounters more antibody, more precipitin is formed. The molecules which become part of the precipitin antigen-antibody complex cease to contribute to the diffusion gradients for free antigen and antibody. Thus, precipitin formation contributes to the reduction of the local concentration of the spreading antigen. When precipitin formation

begins, free antibody is depleted at that site and also begins to diffuse since its concentration is no longer uniform throughout the gel. Its diffusion gradient is toward the disc of immune precipitate expanding around the well. Once the precipitin reaction begins, there is thought to be a relatively narrow zone where antigen diffusing outward meets antibody diffusing inward. This is the zone of precipitin formation and it moves outward as long as the diffusion gradient of free antigen toward the reaction zone exceeds that of free antibody. At some point these gradients become equal and the area of the disc of immune precipitate ceases to expand. Until the reactants are quite depleted, precipitin accumulates at the rim of the disc. This reaction can be used quantitatively since the area of the final disc of precipitin is directly proportional to the amount of antigen placed in the well and inversely proportional to the concentration of antibody initially present in the agar.[177] The results of tests of the reproducibility of quantitative methods similar to the one used in this study have generally been expressed in terms of standard deviation as a percent of the mean. Values in the range 7.5-14% have been obtained.[182, 183]

b. Serum complement in human disease

Average values for whole complement and complement component levels seem to be comparable among the groups of normal subjects who have been studied. There seems to be no variation in whole complement levels with sex[184] or race.[185] A tendency for $C'H_{50}$ to increase with age has been reported.[184]

There are, however, inborn and acquired abnormalities of serum levels of both whole complement and of specific C' components. In these states, serum C' levels may be a small fraction of their normal values. Schur and Austen[168] have tabulated (pp. 4-5) a number of these states with the details of the associated C' system alterations which had been described in the literature through September 1967. These authors also offer the following "presumptive, operative classification on the role of complement in human disease" which names some of the diseases associated with C' system abnormalities and identifies some mechanisms which could be responsible for them:

I. Inborn Errors
   - A. Hereditaty angioneurotic edema.
   - B. C'2 deficiency.
   - C. C'3 abnormalities.
   - D. Agammaglobulinemia with C'1q deficiency.

II. Acquired Abnormalities
   - A. Elevations.
   - B. Depressions.
     1. Increased utilization of complement.
        - a. Fixation of complement by circulating or trapped antigen-antibody complexes (systemic lupus erythematosis, acute glomerulonephritis, subacute bacterial endocarditis, cryoglobulinemia).
        - b. Fixation of complement by antibody to cell or tissue antigens (chronic glomerulonephritis, joint effusions in rheumatoid arthritis, hemolytic anemia).
     2. Decreased synthesis (progressive glomerulonephritis, liver disease).
   - C. Other conditions of unknown presumptive mechanism wherein complement alteration has been noted. (Goodpasture's syndrome, hemolytic anemia, rheumatic fever, myasthenia gravis, graft rejection).

The acqired elevations (II, A. in the outline) of serum com-

plement levels have been associated with a number of inflammatory conditions (e.g. acute rheumatic fever, periarteritis nodosa, acute gout). The C' system has been thought, in these conditions, to behave as an acute phase reactant.

Schur and Austen (II. B. 1. in the outline) and others[186,187] have expressed the hypothesis that the low serum levels of whole complement and of C'1, C'2, C'4, and C'3 observed in diseases believed to be immune in origin are due to fixation of complement components to antigen-antibody complexes. Since β_{1C}-globulin is, in part, fixed on immune precipitates, the demonstration by immunofluorescence of its presence in the renal lesions of systemic lupus erythematosis and acute glomerulonephritis[188] has been interpreted as supportive of the hypothesis that serum C'3 levels fall in those diseases as a result of C'3 fixation. The same findings have also been taken as evidence that the two diseases do, in fact, have an immune pathogenesis. Some authors,[172,189] however, have been reluctant to make much of this hypothesis until more is known about complement metabolism. Recent studies using radioactive iodine labeled C' components do indicate that metabolic factors are important in producing altered serum complement levels in various diseases.[190-193]

In part because blood is a tissue which can be readily sampled, there is considerable information available concerning serum complement and disease. However, serological studies provide a limited view on the entire C' system, which extends beyond the vascular space. It has been recognized[117,163]

that the presence of, or abnormalities of, sequestered or tissue-bound complement might not be reflected in circulating levels. Also, practically nothing is known about the balance of metabolic and excretory factors which maintain or alter serum complement levels. Adjustments of that balance, whether homeostatic or pathologic, could involve shifts of C' components into or out of the circulation.

c. Complement and byssinosis

If a Type III Arthus mechanism is important in the pathogenesis of the acute dust reaction of byssinosis, then it would be expected to involve at least some components of the C' system. There is some evidence, beyond the indirect evidence provided by the knowledge that it fixes to immune complexes and gives rise to an anaphylatoxin, which indicates that β_{1C}-globulin is intimately involved in Arthus reactions. Immunofluorescent tests of skin biopsy specimens taken from sites of intracutaneous Aspergillus fumigatus antigen injections in sensitive subjects showed that "Type III reactions were almost invariably accompanied by the presence of β_1C component of complement and of immunoglobulins in the perivascular cellular infiltration of the skin."[107] In direct passive Arthus reactions produced in rat paws and studied by similar techniques, transient fixation of C'3 to antigen-antibody complexes in the region of small veins, venules, and capillaries was observed. The presence of C'3 in these lesions correlated well with the presence of signs of inflammation.[194]

Presumably with reference to phenomena similar to those reviewed earlier under the heading of the indirect pharmacological hypothesis of acute dust reaction pathogenesis, de Weck and Schneider[195] observed that anaphylactic and Arthus reactions may be closely mimicked by basic charged oligopeptides without, or with only a restricted, participation of specific antigen-antibody interactions. They discuss the possibility that the same chain of molecular events initiated by antigen-antibody interactions may also be initiated unspecifically. The idea is not a new one, but if it is true, then the anaphylatoxin-producing C' components seem good candidates for involvement in the process. Lepow[91] has produced anaphylatoxin by activating C'3 with plasmin and with cobra venom.

d. Goals and limitations

Examination of serum complement levels for changes at the time of acute dust reactions seemed directly relevant to these two possible mechanisms of the production of that reaction. Serum C'3 levels were therefore measured in active and retired hemp workers before and after dust exposure on days when acute dust reactions could be expected. The selection of an immunoassay technique specific for only one component of complement was dictated by practicalities attending the field conditions of the investigation. However, the evidence[174] that C'3 level does not limit the serum hemolytic complement level until it becomes fairly low, coupled with the possibility that C'3 alone could be involved

in a pathogenetic mechanism without affecting other C' components, suggests that the test used may have been the best selection regardless of practical considerations.

The brevity of the acute dust reaction in comparison with human disease states in which C'3 alterations have been observed and the absence of information concerning the regulation of C'3 serum levels rendered the decision of when to obtain post-exposure specimens problematic. In studies of decomplementation in animals, circulating levels have fallen rapidly and have returned to normal, depending upon the species of animal, in from one or two hours to one or two days.[196] With this and similar studies as the only available guides, it was arbitrarily decided to obtain post-exposure specimens fairly soon after dust exposure. One concern was not to miss a rapidly corrected depression of C'3 level.

Since the precision of the method used was such that the standard deviation of multiple measurements of a single sample could not be expected to be much less than 10% of the mean value measured for the sample, it was accepted that subtle changes of C'3 level might be missed. Another factor which could prevent detection of a C'3 alteration by this test is the sensitivity of the test to both β_{1C}-and β_{1A}-globulins. Pondman and Peetoom[197] have reported that β_{1A}-globulin is gradually released following β_{1C}-globulin fixation. Also, there is evidence that the portion of the β_{1C}-globulin molecule lost as it becomes β_{1A}-globulin is active in hemolysis.[198] If the same fragment becomes anaphylatoxin, and

if textile dust acts pharmacologically to split that fragment from β_{1C}-globulin leaving β_{1A}-globulin free in tissue or in the blood, this test would presumably be incapable of detecting change. While anaphylatoxin production has not been described in association with experimental β_{1C}- to β_{1A}-globulin conversion,[197] the conversion fragment might contain the C'3 anaphylatoxin activity and be somehow split fron β_{1C}-globulin by the pharmacological action of textile dust. It seems that either of the routes of conversion mentioned could lead to a replacement of serum β_{1C}-globulin with β_{1A}-globulin which this test would not detect. Free β_{1A}-globulin has been found in fresh serum from patients with active systemic lupus erythematosis.[117]

If C'3 is involved in the acute dust reaction, whether by an allergic or a pharmacological mechanism, there is no assurance that circulating C'3 would even participate in the reaction, much less reveal an altered concentration. Extravascular C'3 alone could be involved. As was indicated earlier, very little is known about the interactions of circulating, extravascular, and newly synthesized C'3.

There are obviously many limitations restricting the conclusions which can be drawn from study of serum C'3 levels in the acute dust reaction. However, the possible findings are limited and all can be interpreted, with appropriate qualifications, in terms of the actual cellular-molecular

mechanisms taking place. If the levels rise, it could be construed as evidence of a pronounced inflammatory response in which the C' system might or might not be involved. If the levels remain the same, again, the C' system might or might not be involved. In the event of either of these two findings, the theoretical reasons for the possible involvement of complement in the acute dust reaction might lead to later investigation of tissue less accessable than the blood. However, before more ambitious studies should be considered, information about circulating complement should be on hand. Lastly, if C'3 levels fall, there would then be more reason to suspect that a mechanism involving complement had occurred. There would not, however, be proof that it had occurred. Yet if decreased circulating C'3 and then perhaps decreased whole serum complement, could be shown to be consistently associated with acute dust reactions, textile plants would become invaluable laboratories for the investigation of C' metabolism needed to remove many of the limitations of inference from this and many other studies.

This investigation of serum C'3 levels was, therefore, undertaken because the C' system may have a role in the pathogenesis of the acute dust reaction. The study did not promise to provide more than a small portion of information about that possible role, but it was the logical place to begin.

2. Precipitating antiboby

The 1964 paper of Massoud and Taylor[42] had reinforced

the inclination of the writer to examine byssinosis from an immunological point of view. The strong anticomplementary activity demonstrated by their antigen preparation fostered the C'3 study because it appeared more likely that textile materials could be involved in complement fixation in vivo or in some other process affecting the C' system. Since sera were going to be obtained, it seemed worthwhile to try to detect precipitating antibody analogous to that reported by those authors. The methodology employed in this study uses double gel diffusion and parallels that which they described. Massoud and Taylor had obtained positive tests for antibody in all the human sera they tested and it was considered sufficient, in seeking to replicate their work in a somewhat different system, to examine a small number of sera. As a refinement on their approach, post-exposure as well as pre-exposure textile worker sera were tested. (Readers may recall from P. 41 that Taylor has recently stated that new evidence indicates that the positive precipitin tests described in 1964 were artifactual.)

Double gel diffusion is a standard antibody detection technique which has numerous forms and other applications.[199-201] It is based on the property of immunological reactants that, when diffusing toward one another in semi-solid media, they react in a zone of immunological equivalence to form a visible precipitin arc or band. For the reaction to occur, neither antigen nor antibody can be present in excess. In antibody detection studies multiple dilutions of the materials

suspected of containing the antigen and the antibody must usually be tested against one another, so that there is opportunity for the condition of equivalence to occur.

Methods

The serologic studies reported here were part of a field study of byssinosis in hemp workers conducted in Callosa de Segura, Spain, during June-August 1967. This work constituted a relatively small portion of the total study, the emphasis of which was on the chronic effects of occupational exposure to hemp dust in a large group of active and retired hemp workers,[25,146] and on acute effects of dust exposure on the respiratory mechanics of thirty-three men recruited from that group plus six members of the research staff.[43] The writer drew bloods from some of the subjects in the respiratory mechanics study before and after their dust exposure. Complement determinations were done on the blood sera in Spain. Later, during November 1967-January 1968, at the John B. Pierce Foundation Laboratory, New Haven, Connecticut, double diffusion tests for precipitating antibody were performed on the sera from a few subjects who seemed to represent a cross-section of those from whom bloods had been obtained. Data from these serologic investigations, which were conducted by the writer, is presented with information about the subjects and their response to dust which was collected by the entire research staff in conjunction with the main portions of the field study. Most of the writer's time in the field was spent collecting such information for the larger investigations: interviewing subjects, conducting basic tests of lung function, and

coaching subjects in the ventilatory maneuvers necessary for the more sophisticated measurements of their respiratory mechanics.

Location and industrial setting. Callosa de Segura has a population of about 14,000 and is within the Province of Alicante. It sits in the valley of the Segura River in southeastern Spain, twenty-two kilometers from the Mediterranian coast. Callosa has long been the principal center in Spain for the processing of soft hemp (Cannabis sativa). In 1947, its hemp industry employed 8,000 people.[25] As the use of synthetic fibers replaced that of soft hemp, the number of active hemp workers diminished dramatically. In 1965, a force of 3,000 workers was in recent memory but only 120 remained.[16] By 1967, the industry had contracted still further. Hemp processing activity was confined to about a dozen one or two-room shops, often located in lofts over private dwellings. Two to ten men worked in each shop. Two larger factories had been in operation in 1965 (factories A and B in reference 16), but one had closed and the other had converted to the production of synthetic fibers.

Respiratory disease in Callosa hemp workers had been studied prior to 1967, both before[15] and after[16] the decline of the industry. Although it was quite apparent at the time of our 1967 study that byssinosis was not likely to be a future problem in Callosa, it was assumed that any increment to the understanding of the disease obtained there would ultimately promote the health of cotton and other textile workers at risk elsewhere and that knowledge gained about chronic respiratory disease might be generally applicable. Further, there were two distinct advantages to studying byssinosis in this rural Spanish town where the hemp industry was disappearing. First, the older segments of the population were stable, so that many people with several years of occupational exposure to hemp dust could be located

with relative ease. Second, the remaining shops were very informally operated. Proprietors were not defensive about being studied and there was no difficulty interrupting work at length for lung function measurements. The shops were, of course, sufficiently dusty to be studied as hazardous work environments. The writer had anticipated a third advantage--that the air quality in Callosa would be such that it could be discounted as a contributor to respiratory disease--but was given reason to question that assumption. Callosa is situated at the base of a small mountain which partially encircles the densely populated center of the town. Two Callosa residents told the writer that in winter the sky is perpetually overcast and that damp, oppressive air seems to become trapped over the town by the mountain. A difficult story to evaluate; but, since the mountain is there (Figures I-III) and there are local sources which would be expected to discharge oxides of sulfur, particulate matter, and other less studied pollutants (these sources include coal-burning locomotives, two and three wheel vehicles driven by two-cycle gasoline engines, automobiles, and trucks), it seems unreasonable to continue to assume that the atmosphere is essentially benign. To the writer's knowledge, quantitative information on air pollution in Callosa is not available.

The raw material of the small hemp factories in Callosa consists of partially processed hemp fibers. Cannabis sativa is sown in the spring and cut in late summer, by which time the slender stalks have reached a height of two to three meters. The cut plants are retted in large pits. After drying, the wood in the stems of the plant is quite brittle and is broken up by passing the entire plant through motor driven fluted rollers. The fibers which remain are the raw material brought to the shops. There they are cleaned of wood chips and other debris and are sorted into long and short lengths. These jobs are accomplished in two stages. First, an espadador, or batter, drapes bundles of

Figures I-III Callosa de Segura

the fibers over an upright board and flails them with a wooden paddle. The sweeping strokes remove most of the wood chips and coarse debris. The espadador also shakes out some of the fine debris and dust (Figure IV) before passing the bundle to the second stage. Rastrilladors, or hacklers, then pull the partially cleaned fibers through an upright comb of sharp steel teeth. This process removes most of the remaining debris and dust from the fibers and, since shorter fibers are caught in the comb as the longer ones are pulled through (Figure V), sorts them by length. Tied in bundles, the sorted fibers are sent elsewhere for further processing and eventual manufacture into rope, fishnets, clothing, etc. Only in factories where hemp fibers are batted and hackled is appreciable dust raised and it is the men who work there (i.e. espadadors and rastrilladors) who are at risk for byssinosis.[15,16]

Subjects. To evaluate the technique of complement determination as performed in the field, the sera of four healthy research staff members were tested. These levels were compared with the manufacturer's normal values. The bloods were drawn in conjunction with dust exposures and pre- and post-exposure levels were measured. Only the pre-exposure levels were used to evaluate the test. Of these four subjects, one (AB) had been briefly exposed to hemp dust previously and another (GK) presented slight evidence of atopic status (reactivity to cats, positive family history for asthma and hay fever).

The twenty men in the serologic study group were either working in the hemp industry as espadadors or rastrilladors or had done so in the past. With one exception (MEM, an active worker studied on a Monday in his own shop), they were all selected from the group of thirty-three men whose respiratory mechanics were studied before and after hemp dust exposure on a Monday or its equivalent (i.e. some ex-workers were studied on other

Figure IV Espadador

Figure V Rastrillador (in foreground)

days of the week, but they had not been in the dusty workrooms for at least two days).[43] The selection of men for serologic study was dictated to some extent by convenience (availability of time and transportation to fully process blood specimens - see below for steps involved) and by the willingness of subjects to undergo venipuncture. An effort was made, however, to include both men who, on the basis of their history and, in some cases, past lung function studies, were expected to react strongly to the dust and men who were expected, for similar reasons, to show no dust reaction.

It had been anticipated that complement levels could be measured in some of the Callosa agricultural workers who served as control subjects in the survey of chronic respiratory disease. Further, early and late morning measurements in a few control subjects would have been desirable. However, none of the agricultural workers were willing to have blood taken. Fortunately, this did not constitute a methodologic disaster. The technique of complement determination in use was well standardized and normal values were available. The normal range was likely derived from tests on people living in the United States, and there may be some question about applying them to people in Spain. However, the one report found with implications for this problem describes an inability to detect differences in hemolytic complement levels across racial and geographic lines.[185] To detect diurnal variation in complement level unrelated to dust exposure, early and late morning specimens were obtained from four workers on a Monday spent away from hemp dust.

Small sums of money were paid to most of the subjects who participated in this study.

Interviews. A Spanish language adaptation of a standard questionnaire[23] (see Appendix) was completed for each of the twenty men in the serologic study group by one of

five interviewers. Most subjects were interviewed as part of the chronic respiratory disease study, days or weeks prior to their monitored dust exposure. A few subjects not seen previously were interviewed on the day of their dust exposure.

The analysis of interview data in this paper treats some of the questionnaire responses in altered or collapsed form. Acknowledgment of sputum production on most days during three months of the year (positive answer to question 7), or a history of chest disease diagnosed by a physician as chronic bronchitis, has been considered presumptive evidence of chronic bronchitis. Byssinosis grades were determined as follows from question 10 for active workers and question 13 for ex-workers: 0.5 = occasional chest tightness on Mondays when working with hemp; 1 = chest tightness or difficulty breathing on every Monday when working with hemp; 2 = chest tightness or difficulty breathing on every Monday and on some other days when working with hemp; 0 = none of these symptoms. Atopic status was assessed by the specific inquiry about bronchial asthma (question 25), the open-ended questions about previous illnesses, and the questions (28-30) concerned with family history. Interviewers attempted to distinguish between symptoms of asthma associated with work in hemp dust and bronchial asthma independent of the dust. Any smoker consuming 50 or more grams of tobacco per week is considered a moderate to heavy smoker.

Dust exposures. Pre-exposure blood specimens and

measurements of lung function were taken from all subjects in the morning, before they had had any contact with hemp dust. Active workers were exposed to dust in the course of their usual Monday morning work. Retired workers were usually at rest in a dusty workroom for two or more hours, but had been instructed to leave the dust if pronounced symptoms occurred. Most exposures in the serological study group ran about three hours. Post-exposure blood specimens and measurements of lung function were taken within 45 minutes of the termination of dust exposure. If on direct questioning subjects reported any sensation of chest tightness, however slight, symptoms were considered present. On the day subjects J-AMF and JNG participated in the serological study they also received placebo tablets as part of a controlled trial of Alupent.[48]

Lung function. Forced expiratory volume in one second ($FEV_{1.0}$) was measured with portable spirometers with transistorized timers (D. Poulton & Son, Barry, Glamorgan, U.K.).[202] The average of the two highest values among five blows was taken as the $FEV_{1.0}$. The absolute changes in $FEV_{1.0}$ during dust exposure have been tabulated rather than the percent change on the assumption that the former set of values are more normally distributed.

Specimens. Approximately 10 ml. of blood was drawn from each subject, with stasis, from an antecubital vein. Twenty guage needles and Red Stopper Vacutainer evacuated glass tubes (Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey) were used. The specimens were refrigerated in the Vacutainer drawing tubes until there was moderate clot reaction. They were then spun in a hand-driven centrifuge and the sera were transferred to polypropylene tubes and stoppered. Pre-exposure sera were refrigerated until post-exposure sera had been obtained. Then all specimens obtained on a given day were packed in ice and transported to a provisional laboratory where small amounts of each specimen were used to set up complement

determinations. The sera were then repacked in ice and transported to a deep freeze where they were accumulated from successive study days. When the field work was complete, all sera were transferred from the deep freeze to an insulated shipping container (Hollinger Corp., Arlington, Virginia) filled with carbon dioxide snow. The snow was renewed with dry ice as necessary while the sera were flown to the United States and transported to New Haven where they were again placed in a deep freeze. Individual specimens were later thawed and tested for precipitating antibody. Specimens were frozen in order to make feasible any indicated additional studies of labile serum factors.

Serum complement determinations. A micro single radial immunodiffusion test for human C'3 (β_{1C}/β_{1A}-globulin) was used (Hyland, Division of Travenol Laboratories, Inc., Los Angeles, California). The manufacturer supplied covered plastic plates with a layer of agar containing specific antibody for human β_{1C}/β_{1A}-globulin and three reference standards containing β_{1C}/β_{1A}-globulin in concentrations of 60, 180, and 330mg/100ml. Six wells were pre-cut in the agar. (See Figure VI; illustration is actual size.)

Usually one plate was used for each run of three unknown specimens. Three of the wells were filled with the three reference standards and each of the remaining wells with the three undiluted serum specimens to be tested in that run. No less than two standards were used on any plate. Serum and standards were placed in the wells using fine glass capillary tubes prepared by heating, drawing out, and then breaking off the narrow end of ordinary capillary syringes. After the wells were filled, the cover was placed on the plates and they were incubated for 16 hours at room temperature in a moist chamber.

After incubation, the diameter of the precipitin discs were measured using a special viewer (also Hyland). (While all serum C'3 levels were determined on the basis of the

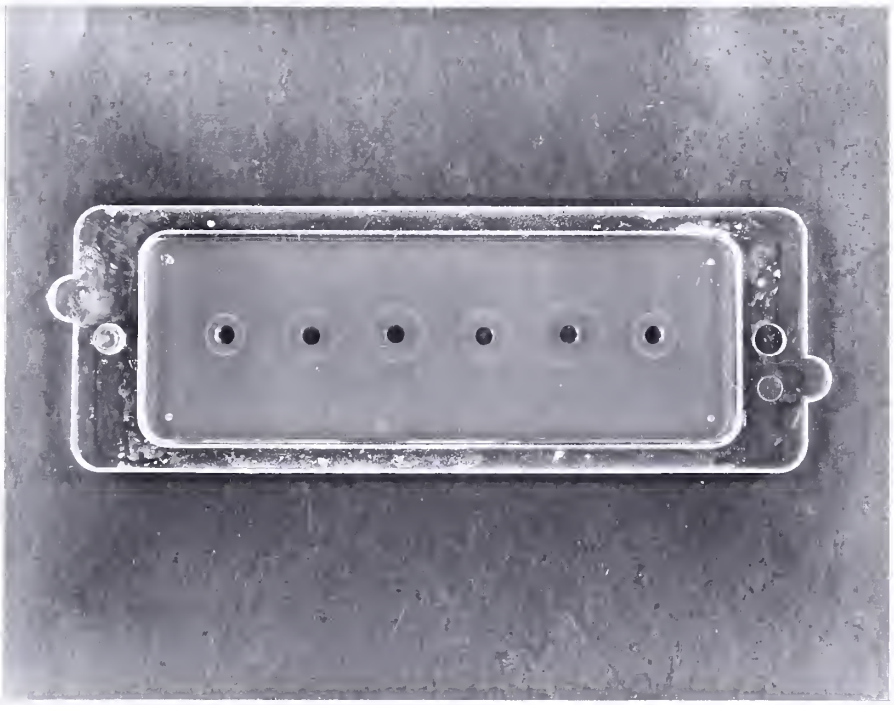


Figure VI Hyland IMMUNO-PLATE of the type used in this study. Standards, in increasing concentration, are in the first, third, and fifth wells from the left.

writer's measurements, a second observer measured the disc diameters on several plates with excellent agreement.) Using two of the cycles of three-cycle semi-logarithmic graph paper, the disc diameters of each of the three standards were plotted on the arithmetic scale versus their concentration on the logarithmic scale. A straight line of best fit was drawn through the three points and the concentration of each unknown specimen in the run was determined from this standard curve. None of the unknowns were outside of the range of the three standards, so no dilutions of serum or standard were necessary.

Duplicate C'3 determinations were done on a number of specimens in order to obtain a measure of the reproducibility of the test. The average of these two values, when obtained, was taken as the C'3 concentration in the specimen.

Test for precipitating antibody. Ouchterlony plates were prepared by pouring 15 ml. quantities of borate buffered 1% agar into plastic Petri dishes and later using a standard template and a vacuum tube to cut a central and six peripheral wells. Precipitin formation was demonstrated in this medium using egg albumin (0.115 mg/ml) and rabbit anti-egg albumin (antiserum diluted 1:6).

Crude antigen preparations were made up as follows:

Extract 1 – 100g of fine hemp dust (collected by the writer in the Callosa work room illustrated in Figure V) extracted overnight with 1 liter normal saline and filtered.

Extract 2 – 50g of Sevillian hemp containing considerable wood and fibers (supplied by a Callosa hemp plant proprietor – local workers reported that they experienced the most severe reactions when working with hemp grown and retted in the region of Spain around Sevilla) extracted overnight with 1 liter normal saline and filtered.

Extract 3 – 20g of cotton bracts (supplied by Dr. A. Bouhuys) were crushed and extracted overnight with 150ml normal saline and filtered.

The filtrates were used undiluted and diluted 1:5, 1:10, and 1:20 with normal saline. Four pair of pre- and post-exposure

sera were selected to include active and ex-workers and strong and marginal dust reactors. These sera, and one fresh normal serum specimen, were used undiluted and diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512 with normal saline. Each concentration of each serum specimen was tested against each concentration of each extract.

Analysis. The statistics text by Snedecor and Cochran[203] was used as a methodological reference and as a source of tables for tests of significance. The t-tests of differences used were all two-tailed.

Information about age, duration of exposure to hemp dust, tobacco consumption, and chronic bronchitis is provided. Some comparisons are made, but readers are referred elsewhere[25,146] for a statistical analysis of these variables based on a much larger group of subjects.

Results

1. Serum complement

a. Staff controls

The pre- and post exposure C'3 levels of four members of the research staff are shown in Table I. Their average pre-exposure C'3 level was 138 mg/100ml. The 95% confidence interval for this value overlaps the mean normal adult level of 145 mg/100ml reported by the manufacturer of the test. The rises in post-exposure levels were not significant at the 5% level of confidence ($t = 1.495$; with 3 d.f., $0.40 > P > 0.20$).

b. Hemp workers

Much of the data collected on the twenty men in the serological study group is summarized in Table II. Most of the column headings are self-explanatory, but a few comments may be helpful. Under Dust Exposure, Sxs refers to whether or not a subject felt chest tightness; $\Delta FEV_{1.0}$ is the signed difference resulting from the subtraction of pre-exposure $FEV_{1.0}$ from post-exposure $FEV_{1.0}$; $\Delta C'3$ results from a similar treatment of C'3 levels. Thus, a minus sign preceding these values indicates a <u>decrease</u> of ventilatory capacity or a <u>decrease</u> of serum C'3 level. Most of the following analysis uses data contained in the table. The questionnaire material is treated first.

<u>Average ages</u>. At the time of the study, the average

Table I

Serum C'3 Levels of Research Staff Members

Before and After Hemp Dust Exposure

Subject	Pre-Exp C'3	Post-Exp C'3
JW	128	134
SK	122	140
GK	119	145
AB	175	182

Units: mg/100ml.

Table II

Summary of Selected Questionnaire Responses of Hemp Workers and Ex-Workers with Symptoms, $\Delta FEV_{1.0}$, and $\Delta C'3$ Associated with Hemp Dust Exposure

Subject	Age	Worker or Ex-Worker	Years in Hemp	Tobacco Consump (Gm/wk)	Chron Sput or Recur. Bronchitis	Byssinosis Grade	Dust Exposure				
							Sxs	$\Delta FEV_{1.0}$	Pre-Exp C'3	Post-Exp C'3	$\Delta C'3$
DAS	49	Worker	34	50-104	yes	1	yes	+0.20	80	90	+10
LBS	39	Worker	21	0	yes	0.5	yes	+0.01	158	132	-26
LSP	52	Worker	37	50-104	no	0	yes	-0.01	132	153	+21
MLS	49	Ex-Worker	35	0	no	0.5	yes	-0.01	140	140	0
AGP	56	Worker	41	0	yes	1	yes	+0.15	160	160	0
MEM	24	Worker	10	50-104	yes	1	yes	+0.05	112	121	+ 9
LPP	47	Ex-Worker	20+	50-104	no	1	yes	-0.57	131	126	- 5
CSM	41	Ex-Worker	26	7-49	yes	1	yes	+0.19	160	145	-15
GMB	31	Ex-Worker	9	105-168	no	1	yes	+0.01	140	150	+10
MTA	25	Ex-Worker	7	0	no	1	no	+0.02	152	139	-13
ACB	31	Worker	18	Ex-Smoker	yes	2	yes	-0.07	164	164	0
LPM	36	Ex-Worker	?	169+	no	1	yes	-0.06	150	176	+16
JSF	32	Ex-Worker	15	Ex-Smoker	yes	1	yes	-0.01	120	115	- 5
J-AMF	27	Worker	13	169+	yes	1	yes	-0.38	140	123	-17
JNG	35	Worker	29	0	no	0	yes	+0.12	120	110	-10
J-MCB	32	Worker	24	0	no	0	no	+0.01	160	144	-16
JML	26	Worker	11	0	no	0	no	-0.06	128	175	+47
FLN	28	Worker	12	0	no	0	no	-0.08	95	104	+ 9
BSM	55	Ex-Worker	35	50-104	yes	2	yes	-0.60	156	156	0
VMR	59	Ex-Worker	28	Ex-Smoker	yes	2	yes	-0.55	127	134	+ 7

Units: $FEV_{1.0}$ in liters, all C'3 values in mg/100ml.

age in years of all the men was 38.7; that of the eleven active workers was 36.3; and that of the nine ex-workers was 41.7.

Atopy. Very few of the subjects gave any history of personal or familial allergic disease. JSF reported bronchial asthma of several years duration. JML stated that both of his parents had had eczema.

Chronic bronchitis. Half the men in the group met the criteria for chronic bronchitis. On the average, these men were older (41.3 vs. 36.1 years) and had worked longer in hemp (24.1 vs. 20.4* years) than the men who did not meet the criteria. Including the ex-smokers on the basis of previous smoking habits (ACB and JSF had been light smokers and VMR, a moderate to heavy smoker), there were five moderate to heavy smokers in the chronic bronchitic group and four not in it.

Effort intolerance. Two of the ex-workers reported marked effort intolerance. CSM answered affirmatively to

*This figure is based on the nine durations of exposure which were approximately known. The duration of exposure entered on the questionnaire of LPM was 3 years, but official records used in drawing samples for the chronic respiratory disease study stated he had worked 15 years in hemp. Neither figure seemed acceptable in the light of the other. The interviewer of subject LPP coded duration of exposure directly rather than recording the actual number of years for coding later. Since 20+ years was checked, 20 was used for this man in calculating the average duration of exposure for the non-bronchitics.

questions 15 through 18 and JSF, to questions 15 and 18. (Question 18 asks if the subject becomes dyspneic when washing or dressing himself.)

Byssinosis grade. Seventy-five percent of the subjects and all the chronic bronchitics met the criteria for some byssinosis grade.

Acute dust reactions. Symptoms of chest tightness and decreases of $FEV_{1.0}$ have been considered indices of acute dust reactions. Ten subjects (DAS, LBS, LSP, LPP, CSM, JSF, J-AMF, JNG, BSM, and VMR) were recorded by a post-exposure interviewer as having experienced chest tightness. This was considered "pronounced" in some cases. Four subjects (MLS, GMB, ACB, and LPM) were recorded as having experienced "slight", "very slight", or "minimal" chest tightness. Two subjects were recorded as having not experienced chest tightness (MTA and J-MCB). At the time Table II was compiled, no record of symptoms or their absence could be found for the remaining four subjects. However, all were active workers studied on Mondays. Two (AGP and MEM) had reported in their initial interview that they always experienced chest tightness on Mondays (byssinosis grade 1); the two others (JML and FLN) had denied experiencing Monday symptoms (byssinosis grade 0). They were classified accordingly.

For all subjects, the average $\Delta FEV_{1.0}$ was -0.082 L. (All $\Delta FEV_{1.0}$ values cited are in liters.) This decrease was not significant at the 5% level ($t = -1.637$; with 19 d.f., $0.20 > P > 0.10$).

The average $\Delta FEV_{1.0}$ for the sixteen men thought to have experienced chest tightness was -0.096. This decrease was not significant at the 5% level ($t = -1.41$; with 15 d.f., $0.20>P>0.10$). The average $\Delta FEV_{1.0}$ for the four men classified as not having experienced chest tightness was -0.028. This was not a significant change ($t = -1.102$; with 3 d.f., $0.50>P>0.40$). The difference between these two average changes in $FEV_{1.0}$ was not significant (pooled estimate of the variance of the difference yielded $t = -0.490$; with 18 d.f.). The average $\Delta FEV_{1.0}$ for the ten men for whom definite chest tightness had been recorded was -0.17. This decrease was significant at the 10% level, but not at 5% ($t = -2.22$; with 9 d.f., $0.10>P>0.05$).

The men who met the criteria for chronic bronchitis had a larger mean decrease in $FEV_{1.0}$ than the other men (-0.101 vs. -0.063), but the difference was not significant (t-test of differences between means of groups of equal size: $t = -0.342$; with 18 d.f.).

Pre-exposure C'3. The average pre-exposure C'3 level in all hemp workers studied (active and ex-workers) was 136 mg/100ml. (All C'3 and ΔC'3 values cited are in mg/100ml.) The 95% confidence interval for this value overlaps the mean normal adult level of 145. These values showed no tendency to be linearly correlated with age ($r = 0.067$). The mean pre-exposure C'3 level of the men classified as chronic bronchitics was 137.7. The difference between this and the somewhat smaller value (134.8) of the other men was

not significant (t-test of differences between means of groups of equal size: t = 0.266; with 18 d.f.).

Though levels found in patients with acute glomerulonephritis and systemic lupus erythematosis are often considerably lower, the pre-exposure β_{1C}/β_{1A}-globulin level measured in one subject (DAS) appeared abnormal. No clinical reason for this could be ascertained.

ΔC'3. The average change of C'3 level (ΔC'3) for all twenty subjects was +1.1 and was not significant (t = 0.301; with 19 d.f.). The average ΔC'3 for the sixteen men with chest tightness was -0.31 and was not significant (t = -0.099; with 15 d.f.). The average for the four men without chest tightness was +6.8 and was not significant (t = 0.521; with 3 d.f.). The ten men with definite records of chest tightness had an average ΔC'3 of -4. This was not significant at the 5% level (t = -0.903; with 9 d.f., $0.40 > P > 0.20$).

There was no significant tendency for ΔC'3 to be linearly correlated with $\Delta FEV_{1.0}$; in the entire group (r = 0.006), in the sixteen men with chest tightness (r = 0.045), in the four men without chest tightness (r = -0.720), or in the ten men with definite chest tightness (r = -0.082).

Control day. The early and late morning (3 hours apart) C'3 levels measured in four subjects on a Monday spent away from hemp dust are shown in Table III. The average ΔC'3 of +3.25 is not significant (t = 0.394; with 3 d.f.). Changes in lung function measurements in these subjects on this day

Table III

Serum C'3 Levels of Hemp Workers on a Monday Spent Away from Hemp Dust

Subject	Early AM C'3	Late AM C'3	ΔC'3
ACB	140	134	- 6
J-AMF	107	122	+15
JNG	128	112	-16
J-MCB	128	148	+20

Units: mg/100ml.

were minimal.[48]

c. Reproducibility of C'3 measurements

Duplicate C'3 determinations were done on eleven specimens. The square root of the value $\Sigma D_i^2/2n$, where the D_i's are the differences between the levels measured for each specimen and n is the number of specimens, yields 10.9 as an estimate of the standard deviation of the individual C'3 determinations in this study. This S.D. was 7.8% of the mean of all levels measured.

2. Precipitating antibody

No precipitating antibody to any antigen preparation used was detected in hemp worker sera or in fresh serum. All Ouchterlony plates were carefully examined after they had sat overnight at room temperature. They were then placed in a moist atmosphere and observed for seven days. No visible precipitin arcs formed.

Discussion

The two serological studies described have not provided evidence to support the hypothesis that the acute dust reaction of byssinosis involves an Arthus type of allergic mechanism or the hypothesis that it involves an indirect pharmacological mechanism mediated by the third component of the complement system. The goal of the serum complement study was to learn whether or not, within the limitations of the technique employed, C'3 levels could be observed to change in association with acute dust reactions. The frequent reports of chest tightness and the trend toward decreases of $FEV_{1.0}$ are evidence that acute dust reactions occurred in the study group. No consistent alteration of C'3 of any remarkable statistical significance was associated with these manifestations of the acute dust reaction. The negative result of the antibody detection study and its consequent inability to support the allergic hypothesis is a straightforward relationship. It should be acknowledged that, in spite of the many combinations of dilutions used, the condition of equivalence may not have been achieved by antigen and specific antibody present in the system.

The information obtained in the complement study suggests that, if serum complement does change during acute dust reactions, very sensitive methods will be necessary to prove it. One approach might be to examine, in a few

subjects, a series of closely spaced post-exposure specimens with the $C'H_{50}$ method and immunoassay methods for C' components which incorporate multiple determinations on each specimen. Dowell and Buckley[204] reported $C'H_{50}$ levels in specimens obtained during elective cardiac catheterizations and noted that similar assays of serum complement activity differences across the pulmonary circulation could provide useful information about inflammatory and vascular lung disease. Their study is cited to indicate that there would be yet more sensitive methods remaining should the suggested approach to further investigation be undertaken. It is not assumed that the method of Dowell and Buckley would ever be applicable to the study of the acute dust reaction.

The present evidence incriminating allergy in byssinosis is not compelling. It is conceivable, however, that allergic mechanisms which are different from any of the classical allergic mechanisms investigated to date could contribute to the pathogenesis of the disease. With time, a mechanism linking the acute and chronic manifestations of byssinosis might even emerge. Secretory antibody, IgA, is present in bronchial secretions and, while little is yet known about the functions of this immunoglobulin, there has been considerable speculation about its possible roles in host defense and in allergic disease.[205,206] In certain chronic respiratory diseases, elevated serum levels of antibody which seems to be IgA and which demonstrates anti-lung activity have been found.[207-209] In chronic bronchitis, qualitative and quanti-

tative changes of immunoglobulins in bronchial secretions have been shown to occur.[210] Since antibody in bronchial secretions seems to have been produced locally by cells which are part of the defense system of the lung, some of the findings described might constitute evidence to further substantiate Green's concept[211] of the pathogenetic determination of chronic bronchitis in which a cellular defect or suppression is the central factor. Green seems to envision failure in the bactericidal activities of these cells. Considering the auto-immune nature of the antibodies mentioned and the age distribution of chronic bronchitis, these findings might also support speculations, similar to those Burnet[212,213] has entertained with regard to cancer, which interrelate ageing, somatic genetics, and immunity.

Conclusion

The studies presented in this paper added investigation of the pathogenesis of byssinosis to the scope of a group of field studies of the epidemiology and pathophysiology of that disease. The logical first step in evaluating the hypothesis that the complement system takes part in the mediation of the acute dust reaction of byssinosis has been carried out. Negative results were obtained and suggestions for further evaluation of the hypothesis have been made. It is hoped that there is now a trend, to which this investigation contributes, toward substantially increasing the knowledge and understanding of host factors in the pathogenesis of byssinosis.

References

1. Ramazzini, B. De Morbis Artificum, The Latin text of 1713, Revised, with translation and notes by W.C. Wright, University of Chicago Press, Chicago, 1940, pp. 256-261.

2. Schilling, R.S.F. "Byssinosis in Cotton and Other Textile Workers", Lancet, 271-I;261-265, 319-325, 1956. (100 references.)

3. Carminita, B.H., Baum, W.F., Neal, P.A., and Schneiter, R. "A Review of the Literature Relating to Affectations of the Respiratory Tract in Individuals Exposed to Cotton Dust", Public Health Bulletin No. 297, U.S. Govt. Printing Office, Washington, D.C., 1947. (267 references.)

4. Smiley, J.A. "Background to Byssinosis in Ulster", Brit. J. Industr. Med., 18:1-9, 1961. (Discursive, but quotes primary sources extensively. 31 references.)

5. Kovats, F. and Bugyi, B. Occupational Mycotic Diseases of the Lung, translated by K. Balazs, Akademiai Kiado, Budapest, 1968.

6. Harpel, G.R. "Byssinosis in Two Southern Cotton Mills", M.P.H. Essay, Dept. of Epidemiology and Public Health, Yale University School of Medicine, 1969. (History of Byssinosis, pp. 3-15, uses 23 references.)

7. Massoud, A. "The Origin of the Term Byssinosis", Brit. J. Industr. Med., 21:168, 1964. (8 references.)

8. Schilling, R.S.F., Vigliani, E.C., Lammers, B., Valic, F., and Gilson, J.C. "A Report on a Conference on Byssinosis", XIVth International Congress of Occupational Health, Vol. II, Madrid, 1963 (International Congress Series, No. 62, Excerpta Medica Foundation, Amsterdam, 1964, pp. 137-145).

9. De Cardona, R. "Bisinosis Versus Cannabosis", Medicina de Empresa, 5:83-85, 1969.

10. Baselga-Monte, M. "Repertorio Bibliographico Sobre Bisinosis y Otras Enfermedades Respiratorias de

los Trabajadores Textiles", Medicina de Empressa, 5:161-172, 1969. (This bibliography, compiled at the Biblioteca Ramazzini de Barcelona, contains 292 references, in chronological order, dated from 1713 through 1968.)

11. Stott, H. "Pulmonary Disease Amongst Sisal Workers", Brit. J. Industr. Med., 15:23-37, 1958.

12. Gilson, J.C., Stott, H., Hopwood, B.E.C., Roach, S.A., McKerrow, C.B., and Schilling, R.S.F. "Byssinosis: The Acute Effect on Ventilatory Capacity of Dusts in Cotton Ginneries, Cotton, Sisal, and Jute Mills", Brit. J. Industr. Med., 19:9-18, 1962.

13. Dantin Gallego, J., Cerra Gonzalez, J., and Comba Ezquerra, G. "Estudio Epidemiologico, Clinico y Funcional Respiratorio del Trabajo del Esparto", Medicina de Empresa, 5:94-105, 1969.

14. Bouhuys, A. "Byssinosis in Textile Workers", Trans. N.Y. Acad. Sci., II, 28:480-490, 1966.

15. Barbero, A. and Flores, R. "Dust Disease in Hemp Workers", Arch. Environ. Health, 14:529-532, 1967. (This paper provides a summary in English of publications by Barbero and Flores which appeared, in Spanish, in 1944, 1948, 1958, and 1963.)

16. Bouhuys, A., Barbero, A., Lindell, S.-E., Roach, S.A., and Schilling, R.S.F. "Byssinosis in Hemp Workers", Arch. Environ. Health, 14:533-544, 1967.

17. Bouhuys, A., Van Duyn, J., and van Lennep, H.J. "Byssinosis in Flax Workers", Arch. Environ. Health, 3:499-509, 1961.

18. Elwood, P.C., Pemberton, J., Merrett, J.D., Carey, G.C.R., and McAulay, I.R. "Byssinosis and Other Respiratory Symptoms in Flax Workers in Northern Ireland", Brit. J. Industr. Med., 22:27-37, 1965.

19. Schilling, R.S.F., Hughes, J.P.W., Dingwall-Fordyce, I., and Gilson, J.C. "An Epidemiological Study of Byssinosis among Lancashire Cotton Workers", Brit. J. Industr. Med., 12: 217-227, 1955.

20. McKerrow, C.B., McDermott, M., Gilson, J.C., and Schilling, R.S.F. "Respiratory Function During

the Day in Cotton Workers: A Study in Byssinosis", Brit. J. Industr. Med., 15:75-83, 1958.

21. Bouhuys, A., Wolfson, R.L., Horner, D.W., Brain, J.D., and Zuskin, E. "Byssinosis in Cotton Textile Workers: Respiratory Survey of a Mill with Rapid Labor Turnover", Ann. Intern. Med., 71:257-269, 1969.

22. Batawi M.A.El, Schilling, R.S.F., Valic, F., and Walford, J. "Byssinosis in the Egyptian Cotton Industry: Changes in Ventilatory Capacity During the Day", Brit. J. Industr. Med., 21: 13-19, 1964.

23. Lammers, B., Schilling, R.S.F., and Walford, J. "A Study of Byssinosis, Chronic Respiratory Symptoms, and Ventilatory Capacity in English and Dutch Cotton Workers, with Special Reference to Atmospheric Pollution", Brit. J. Industr. Med., 21:124-134, 1964.

24. Mekky, S., Roach, S.A., and Schilling, R.S.F. "Byssinosis among Winders in the Cotton Industry", Brit. J. Industr. Med., 24:123-132, 1967.

25. Bouhuys, A., Barbero, A., Schilling, R.S.F. and van de Woestijne, K.P. "Chronic Respiratory Disease in Hemp Workers", Am. J. Med., 46:526-537, 1969.

26. Popa, V., Gavrilescu, N., Preda, N., Teculescu, D., Plecias, M., and Cirstea, M. "An Investigation of Allergy in Byssinosis: Sensitization to Cotton, Hemp, Flax, and Jute Antigens", Brit. J. Industr. Med., 26:101-108, 1969.

27. Schilling, R.S.F.M Hughes, J.P.W., and Dingwall-Fordyce, I. "Disagreement Between Observers in an Epidemiological Study of Respiratory Disease", Brit. Med. J., 1:65-68, 1955.

28. Roach, S.A. and Schilling, R.S.F. "A Clinical and Environmental Study of Byssinosis in the Lancashire Cotton Industry", Brit. J. Industr. Med., 17:1-9, 1960.

29. Arnoldsson, H., Bouhuys, A., and Lindell, S.-E. "Byssinosis: Differential Diagnosis from Bronchial Asthma and Chronic Bronchitis", Acta Medica Scandinavica, 173:761-768, 1963.

30. Belin, L., Bouhuys, A., Hoekstra, W., Johansson, M.-B., Lindell, S.-E., and Pool, J. "Byssinosis in Cardroom Workers in Swedish Cotton Mills", Brit. J. Industr. Med., 22:101-108, 1965.

31. Zuskin, E., Wolfson, R.L., Harpel, G., Welborn, J.W., and Bouhuys, A. "Byssinosis in Carding and Spinning Workers", Arch. Environ. Health, 19:666-673, 1969.

32. McKerrow, C.B. and Schilling, R.S.F. "A Pilot Enquiry into Byssinosis in Two Cotton Mills in the United States", J.A.M.A., 177:850-853, 1961.

33. Valic, F., Zuskin, E., Walford, J., Kersic, W., and Paukovic, R. "Byssinosis, Chronic Bronchitis, and Ventilatory Capacities in Workers Exposed to Soft Hemp Dust", Brit. J. Industr. Med., 25:176-186, 1968.

34. Bouhuys, A. and Lindell, S.-E. letter: "World-wide Byssinosis", Brit. J. Med., 2:1396, 1962.

35. Hill, A.B. "Sickness amongst Operatives in Lancashire Cotton Spinning Mills (with special reference to the Cardroom)", Industrial Health Research Board Report No. 59, H.M.S.O., London, 1930.

36. Schilling, R.S.F. "Byssinosis in the British Cotton Textile Industry", Brit. Med. Bull., 7:52-56, 1950.

37. Schilling, R. and Goodman, N. "Cardiovascular Disease in Cotton Workers: Part I", Brit. J. Industr. Med., 8:77-90, 1951.

38. Schilling, R.S.F. "Byssinosis in the British Cotton Industry", Proc. Roy. Soc. Med., 45: 601-603, 1952.

39. Schilling, R.S.F., Goodman, N., and O'Sullivan, J.G. "Cardiovascular Disease in Cotton Workers Part II: A Clinical Study with Special Reference to Hypertension", Brit. J. Industr. Med., 9: 146-156, 1952.

40. Gernez-Rieux, C., Voisin, C., Jacob, M., Corsin, L., Lefebvre, J. "Enquete Allergologique Chez 40 Ouvriers de Filature de Coton Souffrant de Troubles Dyspneiques au Travail", Le Poumon et le Coeur, 18:651-657, 1962.

41. McKerrow, C.B. and Molyneux, M.K. "Influencia de la Exposicion Previa Sobre los Efectos Agudos Respiratorios de la Inhalacion de Polvo de Algodon", Medicina de Empresa, 5:131-132, 1969.

42. Massoud, A. and Taylor, G. "Byssinosis: Antibody to Cotton Antigens in Normal Subjects and in Cotton Card-Room Workers", Lancet, 7360-II: 607-611, 1964.

43. Taylor, G., Lucas, F., and Massoud, A.A.E. "Immunobiologia de la Bisinosis y Quimica del Antigeno de la Planta del Algodon", Medicina de Empresa, 5:135-136, 1969.

44. Bouhuys, A., Gilson, J.C., and Schilling, R.S.F. "Byssinosis in the Textile Industry: Research, Prevention, and Control", Arch. Environ. Health, 21:475-478, 1970.

45. MacMahon, B. and Pugh, T.F. "Causes and Entities of Disease", in Preventive Medicine, D.W. Clark and B. MacMahon eds., Little, Brown, and Company, Boston, 1967, pp. 11-18.

46. The Workmen's Compensation and Benefit (Byssinosis) Act, 1940 (3&4 Geo. 6, C. 56), in Butterworth's Twentieth Century Statutes, Vol. 37, pp. 252-255.

47. Prausnitz, C. "Investigations on Respiratory Dust Disease in Operatives in the Cotton Industry", Medical Research Council Special Report Series, No. 212, H.M.S.O., London, 1936.

48. Bouhuys, A. and van de Woestijne, K.P. "Respiratory Mechanics and Dust Exposure in Byssinosis", J. Clin. Invest., 49:106-118, 1970.

49. Bouhuys, A. "Airway Dynamics and Broncho-active Agents in Man", in Airway Dynamics: Physiology and Pharmacology, A. Bouhuys ed., Charles C. Thomas, Springfield, Illinois, 1970, pp. 262-282.

50. Gandevia, B. and Milne, J. "Ventilatory Capacity Changes on Exposure to Cotton Dust and Their Relevance to Byssinosis in Australia", Brit. J. Industr. Med., 22:295-304, 1965.

51. Bouhuys, A. "Experimental Studies in Byssinosis", Arch. Environ. Health, 6:742-747, 1963.

52. McKerrow, C.B., Roach, S.A., Gilson, J.C., and

Schilling, R.S.F. "The Size of Cotton Dust Particles Causing Byssinosis: An Environmental and Physiological Study", Brit. J. Industr. Med., 19:1-8, 1962.

53. Bouhuys, A., Hunt, V.R., Kim, B.M., and Zapletal, A. "Maximum Expiratory Flow Rates in Induced Bronchoconstriction in Man", J. Clin. Invest., 48:1159-1168, 1969.

54. Renzetti, A.D., Jones, J.M., Kuschner, M., and Steele, J.D. "Definitions and Classification of Noninfectious Reactions of the Lung", A Statement of the Committee on Diagnostic Standards in Respiratory Diseases of the American Thoracic Society. (reprinted from June 1966 Am. Rev. Resp. Dis. by the National Tuberculosis Association.)

55. Prime, F.J. and Scadding, J.G. letter: "Blood-Gas and Spirometric Findings in Chronic Bronchitis and Asthma", Lancet, I:1372, 1968.

56. van de Woestijne, K.P., Douglas, J.S., and Bouhuys, A. "Pathophysiology of Byssinosis" (abstract), Clin. Res., 16:376, 1968.

57. Zuskin, E. and Valic, F. "Acute Effects of Hemp Dust on Ventilatory Capacity", in Proceedings of the XVth International Congress of Occupational Health, Vienna, 1966, Vol. 3, 291-294. (Paper B XV-6.)

58. Collier, H.O.J. and James, G.W.L. "Humoral Factors in Airway Function, with Particular Reference to Anaphylaxis in the Guinea Pig", in Airway Dynamics: Physiology and Pharmacology, A. Bouhuys ed., Charles C. Thomas, Springfield, Illinois, 1970, pp. 239-252.

59. Houston, J.C., DeNavasquez, S., and Trounce, J.R. "A Clinical and Pathological Study of Fatal Cases of Status Asthmaticus", Thorax, 8:207-213, 1953.

60. Sanerkin, N.G. "Causes and Consequences of Airways Obstruction in Bronchial Asthma", Annals of Allergy, 28:528-538, 1970.

61. Drummond, D.C., Hamlin, M., Donoghue, J.K., and Brownsett, F. "The Effect of Oiling the Cotton on the Airborne Contamination in Card Rooms", Brit. J. Industr. Med., 11:151-155, 1954.

62. Furness, G. and Maitland, H.B. "Studies on Cotton Dust in Relation to Byssinosis Part I: Bacteria and Fungi in Cotton Dust", Brit. J. Industr. Med., 9:138-145, 1952.

63. Drummond, D.G. and Hamlin, M. "Airborne Bacteria in Cotton Mills I: Survey of Counts of Viable Bacteria", Brit. J. Industr. Med., 9:309-311, 1952.

64. Hamlin, M. "Airborne Bacteria in Cotton Mills II: Determination of Types of Viable Bacteria", Brit. J. Industr. Med., 9:311-313, 1952.

65. Maitland, H.B. "Recherches sur le Role de l'Allergie aux Poussieres du Coton dan la Pathogenie de la Byssinose", Revue Medicale de Liege, 11:323-334, 1956.

66. Tuffnell, P. "The Relationship of Byssinosis to the Bacteria and Fungi in the Air of Textile Mills", Brit. J. Industr. Med., 17:304-306, 1960.

67. Douglas, W.W. "Histamine and Antihistamines; 5-Hydroxytryptamine and Antagonists", in The Pharmacological Basis of Therapeutics, L.S. Goodman and A. Gilman eds., The Macmillan Company, New York, 1970, pp. 621-662.

68. Feldberg, W. "Distribution of Histamine in the Body", in Histamine, G.E.W. Wolstenholme and C.M. O'Connor eds., Ciba Foundation Symposium, Little, Brown and Company, Boston, 1956, pp. 4-13.

69. West, G.B. "Histamine and Mast Cells", Ibid., pp. 14-19.

70. MacIntosh, F.C. "Histamine and Intracellular Particles", Ibid., pp. 20-35.

71. Spector, W.G. and Willoughby, D.A. "Endogenous Mechanisms of Injury in Relation to Inflammation", in Cellular Injury, A.V.S. de Reuck and J. Knight eds., Ciba Foundation Symposium, Little, Brown and Company, Boston, 1964, pp. 74-82.

72. Asboe-Hansen, G. "Connective Tissue Response to Injury", in Injury, Inflammation, and Immunity, L. Thomas, J.W. Uhr, and L. Grant eds., Williams and Wilkins Company, Baltimore, 1964, pp. 22-31.

73. Majno, G. "Mechanisms of Abnormal Vascular Permeability

in Acute Inflammation", Ibid., pp. 58-93.

74. Rocha E Silva, M. "The Participation of Substances of Low Molecular Weight in Inflammation with Special Reference to Histamine and Bradykinin", Ibid., pp. 220-241.

75. Selye, H. The Mast Cells, Butterworth Inc., Washington, 1965.

76. Haworth, E. and Macdonald, A.D. "On Histamine in Cotton Dust, and in the Blood of Cotton Workers", J. Hygiene, 37:234-242, 1937.

77. Davenport, A. and Paton, W.D.M. "The Pharmacological Activity of Extracts of Cotton Dust", Brit. J. Industr. Med., 19:19-32, 1962.

78. Nicholls, P.J. "Some Pharmacological Actions of Cotton Dust and Other Vegetable Dusts", Brit. J. Industr. Med., 19:33-41, 1962.

79. Antweiler, H. "New Animal Experiments on the Pathogenesis of Byssinosis and Other Organic Pneumoconioses" (abstract), XIVth International Congress of Occupational Health, Vol. IV, Madrid, 1963 (International Congress Series, No. 62, Excerpta Medica Foundation, Amsterdam, 1964, P. F46).

80. Tuffnell, P. "Experimental Byssinosis", Brit. J. Industr. Med., 17:307-309, 1960.

81. Bouhuys, A., Lindell, S.-E., and Lundin, G. "Experimental Studies on Byssinosis", Brit. Med. J. I:324-326, 1960.

82. Antweiler, H. "Observations About a Histamine Liberating Substance in Cotton Dust", Ann. Occup. Hyg., 2:152-156, 1960.

83. Bouhuys, A. and Lindell, S.-E. "Release of Histamine by Cotton Dust Extracts from Human Lung Tissue In Vitro", Experientia, 17:211-212, 1961.

84. Nicholls, P.J., Nicholls, G.R., and Bouhuys, A. "Histamine Release by Compound 48/80 and Textile Dusts from Lung Tissue In Vitro", in Inhaled Particles and Vapours II, C.N. Davies ed., Pergamon Press, Oxford, 1967, pp. 69-74.

85. Douglas, J., Ridgway, P., and Bouhuys, A. "Efectos Histaminoliberadores por los Componentes de los

Polvos Textiles", Medicina de Empresa, 5:141-142, 1969.

86. Bouhuys, A. and Nicholls, P.J. "The Effect of Cotton Dust on Respiratory Mechanics in Man and in Guinea Pigs", in Inhaled Particles and Vapours II, C.N. Davies ed., Pergamon Press, Oxford, 1967, pp. 75-85.

87. Wassermann, M. "Recherches Experimentales Concernant la Maladie des Fileurs et des Tisseurs de Chanvre", Proceedings of the XII International Congress on Occupational Health, Helsinki, 1957, Vol. III, pp. 374-378.

88. Cavagna, G., Foa, V., and Vigliani, E.C. "Consecuencias Cronicas Anatomopatologicas, Immunologicas y Funcionales en el Conejo por la Inhalacion de Extractos de Algodon y Endotoxinas Purificadas", Medicina de Empresa, 5:139-141, 1969.

89. Antweiler, H. "Accion del Polvo de Algodon y de sus Extractos Sobre la Funcion Respiratoria de Gatos y Cobayos", Medicina de Empresa, 5:133-134, 1969.

90. Pernis, B., Vigliani, E.C., Cavagna, C., and Finulli, M. "The Role of Bacterial Endotoxins in Occupational Diseases Caused by Inhaling Vegetable Dusts", Brit. J. Industr. Med., 18: 120-129, 1961.

91. Lepow, I.H. "Complement as a Mediator of Cytotoxicity and Inflammation", Guest Lecture, Dept. of Pathology, Yale University School of Medicine, October 30, 1967.

92. Lepow, I.H., Dias Da Silva, W., and Eisele, J.W. "Nature and Biological Properties of Human Anaphylatoxin", in Biochemistry of the Acute Allergic Reactions, K.F. Austen and E.L. Becker eds., F.A. Davis Company, Philadelphia, 1968, pp. 265-282.

93. Werner, G.C.H. "Essai d'un Nouvel Antihistaminique dan la Byssinose", Archives des Maladies Professionnelles, 25:123-126, 1964.

94. Cavagna, G. "The Endotoxin Content of Vegetable Dusts", XIVth International Congress of Occupational Health, Vol. II, Madrid, 1963 (International Congress Series, No. 62, Excerpta Medica Foundation, Amsterdam, 1964, pp. 693-697).

95. Vigliani, E.C. "Advances in the Pathogenesis of Some Occupational Diseases", Ibid., pp. 126-136.

96. Antweiler, H. "Histamine Liberation by Cotton Dust Extracts: Evidence Against its Causation by Bacterial Endotoxins", Brit. J. Industr. Med., 18:130-132, 1961.

97. Cavagna, G., Foa, V., Nichelatti, T., Calgaro, M., and Locati, G. "Hypersensitivity Reaction to the Endotoxins Contained in Vegetable Dusts", in Proceedings of the XVth International Congress of Occupational Health, Vienna, 1966, Vol. 3, 299-304. (Paper B XV-10.)

98. Heaphy, L.J. and Kilburn, K.H. "Byssinosis: A Model of Airway Reactivity in Man" (abstract), Am. Rev. Resp. Dis., 92:329, 1965.

99. Wuthrich, B. "Zur Byssinose (Baumwollstaublunge) - Handelt es sich um eine Arthus-Reaktion?", Schweiz. Med. Wschr., 99:1041-1051, 1969.

100. Bouhuys, A. "Response to Inhaled Histamine in Bronchial Asthma and in Byssinosis", Am. Rev. Resp. Dis., 95:89-93, 1967.

101. Bouhuys, A. "Prevention of Monday Dyspnea in Byssinosis: A Controlled Trial with an Antihistamine Drug", Clin. Pharm. Therapeut., 4:311-314, 1963.

102. Austen, K.F. "Histamine and Other Mediators of Allergic Reactions", in Immunological Diseases, M. Samter ed., Little, Brown and Company, Boston, 1965, pp. 211-225.

103. Brocklehurst, W.E. "A Slow Reacting Substance in Anaphylaxis - 'SRS-A'", in Wolstenholme and O'Connor, Op. cit., pp. 175-179.

104. Brocklehurst, W.E. "Kinin and Kinin-Forming Enzymes in Anaphylaxis", in Austen and Becker, Op. cit., pp. 297-303.

105. Brocklehurst, W.E. "Pharmacological Mediators of Hypersensitivity Reactions", in Clinical Aspects of Immunology, P.G.H. Gell and R.R.A. Coombs eds., Blackwell Scientific Publications, Oxford, 1968, pp. 611-632.

106. Coombs, R.R.A. and Gell, P.G.H. "Classification of Allergic Reactions Responsible for Clinical Hypersensitivity and Disease", Ibid., pp. 575-596.

107. Pepys, J. "Hypersensitivity Diseases of the Lungs Due to Fungi and Organic Dusts", S. Karger, Basel, 1969.

108. Ishizaka, K. and Ishizaka, T. "Human Reaginic Antibodies and Immunoglobulin E", J. Allergy, 42:330-363, 1968.

109. Ishizaka, K. "The Identification and Significance of Gamma E", Hosp. Pract., 4:70-81, 1969.

110. Johansson, S.G.O. "Raised Levels of a New Immunoglobulin Class (IgND) in Asthma", Lancet, II (7523):951-953, 1967.

111. Wide, L., Bennich, H., Johansson, S.G.O. "Diagnosis of Allergy by an In-Vitro Test for Allergen Antibodies", Lancet, II (7526):1105-1107, 1967.

112. Schwartz, D.P. and Buckley, R.H. "Serum IgE Concentrations and Skin Reactivity to Anti-IgE Antibody in IgA-Deficient Patients", N.E.J.M., 284:513-517, 1971.

113. Frankland, A.W. "The Pathogenesis of Asthma, Hay Fever and Atopic Diseases", in Gell and Coombs, Op. cit., pp. 633-659.

114. Schild, H.O. "Biochemical and Immunological Mechanisms of Allergic Disease", in Bouhuys, Op. cit. (ref. 58), pp. 229-238.

115. Keller, R. "Tissue Mast Cells in Immune Reactions", American Elsevier Publishing Co., New York, 1966.

116. Schild, H.O. "Mechanism of Anaphylactic Histamine Release", in Austen and Becker, Op. cit., pp. 99-118.

117. Iachmann, P.J. "Complement", in Gell and Coombs, Op. cit., pp. 384-419.

118. Ruddy, S. "Natural Control Mechanisms of the Complement System", Guest Seminar, Dept. of Microbiology, Yale University School of Medicine, February 26, 1971.

119. Slomka, M.B. and Goth, A. "Some Observations on the Nature of Refractoriness to Histamine Liberators", Proc. Soc. Exper. Biol. Med., 93:30-32, 1956.

120. Pepys, J. "Skin Tests in Diagnosis", in Gell and

Coombs, Op. cit., pp. 189-220.

121. Cayton, H.R., Furness, G., and Maitland, H.B. "Studies on Cotton Dust in Relation to Byssinosis Part II: Skin Tests for Allergy with Extracts of Cotton Dust", Brit. J. Industr. Med., 9:186-196, 1952.

122. Cayton, H.R., Furness, G., Jackson, D.S., and Maitland, H.B. "Studies on Cotton Dust in Relation to Byssinosis Part III: Comparison of Cotton Dust and House Dust by Chemical and Skin Tests", Brit. J. Industr. Med., 9:303-308, 1952.

123. Bouhuys, A. "Response to Inhaled Histamine in Bronchial Asthma and in Byssinosis", Am. Rev. Resp. Dis., 95:89-93, 1967.

124. Massoud, A.A.E., Altounyan, R.E.C., Howell, J.B.L., and Lane, R.E. "Effects of Histamine Aerosol in Byssinotic Subjects", Brit. J. Industr. Med., 24:38-40, 1967.

125. Wolfromm, R. "Allergie Respiratoire aux Textiles", C.R. Congres des Societes Savantes de Paris, 1956, pp. 67-76.

126. Lopez Merino, Romar Mico, Llopis Llombart, Flores Marco, and Barbero Carnacero "Aspectos Alergicos en los Trabajadores de la Industria del Canamo", Enfermedades del Torax, 15:955-958, 1966.

127. Massoud, A.A.E., Taylor, G., and Lucas, F. "Accion Bronquial en Bisinoticos del Antigeno de la Planta de Algodon", Medicina de Empresa, 5:136-138, 1969.

128. Douglas, J. personal communication.

129. Faux, J.A., Holford-Strevens, V., Wells, I.D., and Pepys, J. "'False Positive' Precipitation Reactions to Extracts of Organic Dusts Due to a Teichoic Acid from S. Aureus", Clin. Exp. Immunol., 7:897-902, 1970.

130. MacIntosh, F.C. and Paton, W.D.M. "The Liberation of Histamine by Certain Organic Bases"; J. Physiol., 109:190-219, 1949.

131. Keller, R. "On the Role of Secondary Mast-Cell Damage and Histamine Release in the Course of Immune Reactions", in Austen and Becker, Op. cit., pp. 253-264.

132. Meneely, G.R., Renzetti, A.D., Steele, J.D., Wyatt, J.P., Harris, H.W. "Chronic Bronchitis, Asthma, and Pulmonary Emphysema", A Statement by the Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases of the American Thoracic Society, Am. Rev. Resp. Dis., 85:762-768, 1962.

133. Heard, B.E. Pathology of Chronic Bronchitis and Emphysema, J.&A. Churchill Ltd., London, 1969.

134. Cassell, E.J. "New and Emergent Diseases", in Human Ecology and Public Health, E.D. Kilbourne and W.G. Smillie eds., The Macmillan Co., Toronto, 1969, pp. 308-359.

135. McKusick, V.A., Goodman, R.M., and Danks, D.M. "Genetic Aspects of Respiratory Disease", Arch. Environ. Health, 6:25-36, 1963.

136. Eriksson, S. "Studies in α_1-Antitrypsin Deficiency", Acta Medica Scandinavica, Suppl. 432:1-85, 1965.

137. Goldsmith, J.R. "Epidemiology of Chronic Bronchitis and Emphysema I. Factors Influencing Prevalence and a Criterion for Testing their Interaction", in The Pathogenesis of the Chronic Obstructive Bronchopulmonary Disease, R.S. Mitchell ed., S. Karger, New York, 1965, pp. 1-23.

138. Advisory Committee to the Surgeon General of the Public Health Service "Relation of Smoking to Diseases of the Respiratory System", in Chronic Diseases and Public Health, A.M. Lilienfeld and A.J. Gifford eds., The Johns Hopkins Press, Baltimore, 1966, pp. 712-743.

139. Ferris, B.G. "Epidemiological Studies on Air Pollution and Health", Arch. Environ. Health, 16:541-555, 1968.

140. Winkelstein, W. and Kantor, S. "Respiratory Symptoms and Air Pollution in an Urban Population of Northeastern United States", Arch. Environ. Health, 18:760-767, 1969.

141. Speizer, F.E. "An Epidemiological Appraisal of the Effects of Ambient Air on Health: Particulates and Oxides of Sulfur", J. Air Pollution Control Assoc., 19:647-655, 1969.

142. Higgins, I.T.T. "An Approach to the Problem of Bronchitis in Industry: Studies in Agricultural, Mining, and Foundry Communities", in Industrial Pulmonary Diseases, E.J. King and C.M. Fletcher eds., J.A. Churchill Ltd., London, 1960, pp. 195-207.

143. De Boer, W.G.R.M. "Byssinosis", Med. J. Aust., II: 1040-1041, 1966.

144. Dingwall-Fordyce, I. and O'Sullivan, J.G. "Byssinosis in the Cotton Waste Industry", Brit. J. Industr. Med., 23:53-57, 1966.

145. Merlo, G. and Monteverde, A. "Fotoendoscopia dei Bronchi nell'Asbestosi, nella Bissinosi, nella Pneumopatia dell'Agricoltore e da Canapa", Medicina del Lavoro, 54:737-741, 1963.

146. Bouhuys, A., Schilling, R.S.F., and van de Woestijne, K.P. "Cigarette Smoking, Occupational Dust Exposure, and Ventilatory Capacity", Arch. Environ. Health, 19:793-797, 1969.

147. Theodore, J., Robin, E.D., and Gee, J.B.L. "Lung Energetics and Lung Disease", in Current Research in Chronic Airways Obstruction, Ninth Aspen Emphysema Conference (1966), Public Health Service Publication 1717, 1968, pp. 3-11.

148. Hogg, J.C., Macklem, P.T., and Thurlbeck, W.M. "Site of Airway Obstruction in Chronic Obstructive Lung Disease", N.E.J.M., 278:1355-1360, 1968.

149. Macklem, P.T., Thurlbeck, W.M., and Fraser, R.G. "Chronic Obstructive Disease of Small Airways", Ann. Intern. Med., 74:167-177, 1971.

150. Mead, J., Turner, J.M., Macklem, P.T., and Little, J.B. "Significance of the Relationship Between Lung Recoil and Maximum Expiratory Flow", J. Appl. Physiol., 22:95-108, 1967.

151. Nadel, J.A. and Comroe, J.H. "Acute Effects of Inhalation of Cigarette Smoke on Airway Conductance", J. Appl. Physiol., 16:713-716, 1961.

152. McDermott, M. and Collins, M.M. "Acute Effects of Smoking on Lung Airways Resistance in Normal and Bronchitic Subjects", Thorax, 20:562-569, 1965.

153. Robertson, D.G., Warrell, D.A., Newton-Howes, J.S., and Fletcher, C.M. "Bronchial Reactivity to Cigarette and Cigar Smoke", Brit. Med. J., III:269-271, 1969.

154. Guyatt, A.R., Berry, G., Alpers, J.H., Bramley, A.C., and Fletcher, C.M. "Relationship of Airway Conductance and Its Immediate Change on Smoking to Smoking Habits and Symptoms of Chronic Bronchitis", Am. Rev. Resp. Dis., 101:44-54, 1970.

155. Clarke, B.G., Guyatt, A.R., Alpers, J.H., Fletcher, C.M., and Hill, I.D. "Changes in Airway Conductance on Smoking a Cigarette", Thorax, 25: 418-422, 1970.

156. Bates, D.V. "Chronic Bronchitis and Emphysema", N.E.J.M., 278:546-551; 600-605, 1968.

157. Innes, I.R. and Nickerson, M. "Drugs Acting on Postganglionic Adrenergic Nerve Endings and Structures Innervated by Them (Sympathomimetic Drugs)", in Goodman and Gilman, Op. cit. (ref. 67), pp. 478-523.

158. Tiffeneau, R. and Beauvallet, M. "Role Respectif des Processus Broncho-moteurs et Vaso-moteurs dans la Production de la Crise D'Asthme", Paris Medical, 129:59-60, 1945.

159. Bouhuys, A., Hartogensis, F., and Korfage, H.J.H. "Byssinosis Prevalence and Flax Processing", Brit. J. Industr. Med., 20:320-323, 1963.

160. McDermott, M., Nicholls, P., Edwards, J., and Skidmore, J. "Acciones Fisiologicas Immunobiologicas y Farmacologicas por Inhalacion de Polvo de Algodon", Medicina de Empresa, 5:138-139, 1969.

161. Muller-Eberhard, H.J. "Chemistry and Reaction Mechanisms of Complement", Adv. Immunol., 8:1-80, 1968. (298 references.)

162. Complement Workshop: "Nomenclature of Complement", Immunochemistry, 3:495, 1966.

163. Lepow, I.H. "Serum Complement and Properdin", in Samter, Op. cit. (ref. 102), pp. 188-210. (140 references.)

164. Rosenberg, L.T. "Complement", Ann. Rev. Microbiol., 19:285-300, 1965. (186 references.)

165. Wolstenholme, G.E.W. and Knight, J. eds. Complement, Ciba Foundation Symposium, Little, Brown and Company, Boston, 1965.

166. Osler, A.G "Functions of the Complement System", Adv. Immunol., 1:131-210, 1961. (630 references.)

167. Kabat, E.A. and Mayer, M.M. Experimental Immuno-Chemistry, Charles C. Thomas, Springfield, Illinois, 1961.

168. Schur, P.H. and Austen, K.F. "Complement in Human Disease", Ann. Rev. Med., 19:1-24, 1968.

169. Muller-Eberhard, H.J., Nilsson, U., and Aronsson, T. "Isolation and Characterization of Two β_1-Glycoproteins of Human Serum", J. Exp. Med., 111:201-215, 1960.

170. Muller-Eberhard, H.J. and Nilsson, U. "Relation of a β_1-Glycoprotein of Human Serum to the Complement System", J. Exp. Med., 111:217-234, 1960.

171. Nilsson, U.R. and Muller-Eberhard, H.J. "Isolation of β_{1F}-Globulin from Human Serum and Its Characterization as the Fifth Component of Complement", J. Exp. Med., 122:277-298, 1965.

172. Gotoff, S.P., Fellers, F.X., Vawter, G.F., Janeway, C.A., and Rosen, F.S. "The $Beta_{1C}$ Globulin in Childhood Nephrotic Syndrome", N.E.J.M., 273: 524-529, 1965.

173. Blaker, F., Fischer, K., and Witte, P. "Quantitative Complement Analyses (C'3) with Radial Immunodiffusion on Patients with Disturbed Immunological State", Dtsch. Med. Wschr., 94:1978-1982, 1969 (abstracted in Excerpta Medica, Section 26, "Immunology, Serology, and Transplantation", 4:480, 1970).

174. Klemperer, M.R., Gotoff, S.P., Alper, C.A., Levin, A.S., and Rosen, F.S. "Estimation of the Serum $Beta_{1C}$ Globulin Concentration: Its Relation to the Serum Hemolytic Complement Titer", Pediatrics, 35:765-769, 1965.

175. Petrie, G.F. "A Specific Precipitin Reaction Associated with the Growth on Agar Plates of Meningococcus, Pneumococcus, and B. Dysenteriae (Shiga)", Brit. J. Exp. Path., 13:380-394, 1932.

176. Ouchterlony, O. "Antigen-Antibody Reactions in Gels", Acta Pathol. Microbiol. Scand., 26:507-515, 1949. (See also the adjacent - pp. 516-524 - paper by the same author.)

177. Mancini, G., Carbonara, A.O., and Heremans, J.F. "Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion", Immunochemistry, 2:235-254, 1965.

178. Lou, K. and Shanbrom, E. "Immunodiffusion Techniques in Clinical Medicine II. Radial Immunodiffusion", J.A.M.A., 200:323, 1967.

179. Crowle, A.J. "Four Modifications of the Micro Agar Diffusion Precipitin Test", J. Lab. Clin. Med., 55:593-604, 1960.

180. Spiers, J.A. and Augustin, R. "Antigen-Antibody Reactions in Gel Single Diffusion: Theoretical Considerations", Transactions of the Faraday Society, 54:287-295, 1958.

181. Augustin, R., Hayward, B.J., and Spiers, J.A. "Antigen-Antibody Reactions in Agar I. Experimental Tests of Current Theories of Oudin's Single Diffusion Method", Immunology, 1:67-80, 1958.

182. Fahey, J.L. and McKelvey, E.M. "Quantitative Determination of Serum Immunoglobulins in Antibody-Agar Plates", J. Immunol., 94:84-90, 1965.

183. Collins-Williams, C., Toft, B., Generoso, L., and Moscarello, M. "Quantitative Immunoglobulin Levels (IgG, IgA, and IgM) in Children, Determined by the Hyland Immunoplate Technique", Canad. Med. Assn. J., 96:1510-1513, 1967.

184. Papanayiotou, P., Papaniotou, K., Papavassiliou, J., and Mastoraki, D. "Physiologic Variation in Serum Complement. Influence of Age and Sex on Levels of $C'H_{50}$", Ann. Inst. Pasteur, 117: 796-800, 1969 (abstracted in Excerpta Medica, Section 26, "Immunology, Serology, and Transplantation", 4:518, 1970).

185. Linhard, J. "Titrage du Complement Chez L'Africain Normal", Bull. Soc. Pathol. Exotique, 55:980-984, 1962.

186. West, C.D., Northway, J.D., and Davis, N.C. "Serum Levels of $Beta_{1C}$ Globulin, a Complement Component,

in the Nephritides, Lipoid Nephrosis, and Other Conditions", J. Clin. Invest., 43:1507-1517, 1964.

187. Dixon, F.J. "The Role of Antigen-Antibody Reactions in Localized Tissue Injury", in Thomas, Uhr, and Grant, Op. cit. (ref. 72), pp. 257-263.

188. Lachmann, P.J., Muller-Eberhard, H.J., Kunkel, H.G., and Paronetto, F. "The Localization of In Vivo Bound Complement in Tissue Sections", J. Exp. Med., 115:63-82, 1962.

189. Tina, L.U., D'Albora, J.B., Antonovych, T.T., Bellanti, J.A., and Calcagno, P.L. "Acute Glomerulonephritis Associated with Normal Serum β_{1C}-Globulin", Amer. J. Dis. Child, 115:29-36, 1968.

190. Alper, C.A., Levin, A.S., and Rosen, F.S. "Beta-1C-Globulin: Metabolism in Glomerulonephritis", Science, 153:180-182, 1966.

191. Alper, C.A. and Rosen, F.S. "Studies of the In Vivo Behavior of Human C'3 in Normal Subjects and Patients", J. Clin. Invest., 46:2021-2034, 1967.

192. Petz, L.D., Fink, D.J., Letsky, E.A., Fudenberg, H.H., and Muller-Eberhard, H.J. "In Vivo Metabolism of Complement I. Metabolism of the Third Component (C'3) in Acquired Hemolytic Anemia", J. Clin. Invest., 47:2469-2484, 1968.

193. Carpenter, C.B., Ruddy, S., Shehadeh, I.H., Muller-Eberhard, H.J., Merrill, J.P., and Austen, K.F. "Complement Metabolism in Man: Hypercatabolism of the Fourth (C4) and Third (C3) Components in Patients with Renal Allograft Rejection and Hereditary Angioedema (HAE)", J. Clin. Invest., 48:1495-1505, 1969.

194. Denk, H., Forster, O., and Kraft, D. "Third Complement Component (C'3) in the Paw of the Rat during the Arthus Reaction", Z. Immun. Forsch. Allerg. Klin. Immun., 138:169-177, 1969 (abstracted in Excerpta Medica, Section 26, "Immunology, Serology, and Transplantation", 4:141, 1970).

195. de Weck, A.L. and Schneider, C.H. "Some Observations on the Molecular Mechanisms of Immediate-type Allergic Reactions", Int. Arch. Allergy, 36:62-74, 1969.

196. Seltzer, G., Baron, S., and Fusco, J. "A Method for Removing Complement In Vivo and Its Rate of Return", J. Immunol., 69:367-372, 1952.

197. Pondman, K.W. and Peetoom, F. "The Significance of the Antigen-Antibody Complement Reaction - IV. The Transformation of β_1C-Globulin Into β_1A-Globulin", Immunochemistry, 1:65-90, 1964.

198. West, C.D., Davis, N.C., Forristal, J., Herbst, J., and Spitzer, R. "Antigenic Determinants of β_{1C}- and β_{1G}-Globulins", J. Immunol., 96:650-658, 1966.

199. Crowle, A.J. Immunodiffusion, Academic Press, New York, 1961.

200. Ouchterlony, O. "Diffusion-In-Gel Methods for Immunological Analysis II", Progr. Allergy, 6:30-154, 1962.

201. Ouchterlony, O. "Gel-Diffusion Techniques", in Immunological Methods, J.A. Ackroyd ed., Blackwell Scientific Publications, Oxford, 1964.

202. McKerrow, C.B., McDermott, M., and Gilson, J.C. "A Spirometer for Measuring the Forced Expiratory Volume with a Simple Calibrating Device", Lancet, I: 149-151, 1960.

203. Snedecor, G.W. and Cochran, W.G. Statistical Methods, The Iowa State University Press, Ames, Iowa, 1967.

204. Dowell, A.R. and Buckley, C.E. "Arteriovenous Complement Activity Differences across the Human Lesser Circulation", Am. Rev. Resp. Dis., 102: 213-220, 1970.

205. Tomasi, T.B. and Bienenstock, J. "Secretory Immunoglobulins", Adv. Immunol., 9:1-96, 1968.

206. Collins-Williams, C., Lamenza, C., and Nizami, R. "Immunoglobulin IgA: A Review of the Literature", Annals of Allergy, 27:225-231, 1969.

207. Hagadorn, J.E. and Burrell, R. "Lung-Reactive Antibodies in IgA Fractions of Sera from Patients with Pneumoconiosis", Clin. Exp. Immunol., 3:263-267, 1968.

208. Lewis, D.M., Lapp, N.L., and Burrell, R. "Quantification of Secretory Immunoglobulin A in

Chronic Pulmonary Disease", Am. Rev. Resp. Dis., 101:55-61, 1970.

209. Esber, H.J. and Burrell, R.G. "Further Characterization of the Specificity of Lung-Reactive Antibodies in Human Serum", Arch. Environ. Health, 21:502-507, 1970.

210. Zedda, S., Amante, L., and Ambrosi, L. "Immunologic Study of the Sputum of Chronic Bronchitis", Med. Lavoro (Milano), 60:350-359, 1969 (abstracted in Excerpta Medica, Section 26, "Immunology, Serology, and Transplantation", 4:400, 1970).

211. Green, G. "Cell Dysfunction as Pathogenetic Determinant in Chronic Bronchopulmonary Disease", Arch. Environ. Health, 21:481-482, 1970.

212. Burnet, F.M. The Integrity of the Body, The Harvard University Press, Cambridge, 1963.

213. Burnet, F.M. "A Modern Basis for Pathology", Lancet, 7557-I:1383-1387, 1968.

Appendix

John B. Pierce Foundation Laboratory
Yale University School of Medicine
New Haven, Connecticut

CUESTIONARIO SOBRE SINTOMATOLOGIA RESPIRATORIA

NOMBRE _____
Apellidos

_____
Nombre propio

FABRICA _____

DOMICILIO _____

_____

_____

Fecha de nacimiento _____
Día *mes* *Año*

Fecha de la entrevista _____
Día *mes* *Año*

NUMERO ☐☐☐☐ 1-4

_____

For coding later ☐ 5

Survey ☐☐ 6-7

Fecha de la entrevista ☐☐ 8-9

Entrevistador ☐☐ 10-11

Domicilio ☐☐ 12-13

Edad ☐☐ 14-15

RACE

Caucasian	0 ☐	16
Negro	1 ☐	
Oriental	2 ☐	
Other	3 ☐	

CIVIL STATE

Single	0 ☐	17
Married	1 ☐	
Widowed	2 ☐	
Div./Sep.	3 ☐	

SEX

Male	0 ☐	18
Female	1 ☐	

SELECTION

Worker at risk	0 ☐	19
Control	1 ☐	
Ex-worker	2 ☐	

TOS

1. ¿ Tose Vd. usualmente nada más de despertarse por las mañanas?
   No 0 ☐
   Yes 1 ☐

2. ¿ Tose Vd. usualmente durante el día o durante la noche?
   No 0 ☐
   Yes 1 ☐

3. ¿ Tose Vd. así la mayoría de los días y durante un período de tiempo de 3 meses cada año?
   No 0 ☐ 20
   Yes 1 ☐

4. ¿ Tose Vd. algún día de la semana en particular?
   No 0 ☐ 21
   Solo los lunes, alguna vez 1 ☐
   Solo los lunes, siempre 2 ☐
   Lunes y Martes 3 ☐
   Lunes y otros días de trabajo 4 ☐
   Días otros que lunes 5 ☐

EXPECTORACION o otra palabra usada localmente (por ejemplo arrancar)

5. ¿ Expectora Vd. usualmente nada más de despertarse por las mañanas y la expectoración proviene de su pecho?
   No 0 ☐
   Yes 1 ☐

6. ¿ Expectora Vd. usualmente durante el día o durante la noche y la expectoración proviene de su pecho?
   No 0 ☐
   Yes 1 ☐

7. ¿ Expectora Vd. así la mayoría de los días y durante un período de tiempo de 3 meses cada año?
   No 0 ☐ 22
   Yes 1 ☐

8. ¿ Ha expectorado Vd. sangre alguna vez?
   No 0 ☐ 23
   Yes 1 ☐

OPRESION EN EL PECHO

9. ¿ Siente Vd. alguna vez opresión en el pecho o dificultad para respirar?
   No 0 ☐ 24
   Yes 1 ☐

10. ¿ Siente Vd. opresión en el pecho o dificultad para respirar algún día de la semana en particular?
    No 0 ☐ 25
    Solo los lunes, alguna vez 1 ☐
    Solo los lunes, siempre 2 ☐
    Lunes y Martes 3 ☐
    Lunes y otros días de trabajo 4 ☐
    Días otros que lunes 5 ☐

11. ¿ A qué hora siente Vd. la opresión en el pecho los lunes? ☐☐ 26-27
    desde _____ a _____ no lo sabe
    Code later

12. ¿ A qué hora siente Vd. la opresión en el pecho los martes? ☐☐ 28-29
    desde _____ a _____ no lo sabe
    Code later

13. Si la respuesta a la pregunta 10 es negativa:
¿ Ha sentido Vd. alguna vez opresión en el pecho o dificultad para respirar algún día de la semana en particular?

No	0 ☐	30
Solo los lunes, alguna vez	1 ☐	
Solo los lunes, siempre	2 ☐	
Lunes y Martes	3 ☐	
Lunes y otros días de trabajo	4 ☐	
Días otros que lunes	5 ☐	

DISNEA o otra palabra usada localmente (por ejemplo fatiga)

14 Si la incapacidad del sujeto para la marcha obedece a otra causa que no sea una enfermedad del corazón o de los pulmones, marque con una 0 el recuadro y no haga las preguntas 15-18. Si no, marque con una 1 y sigue. ☐ 31

LOS FINES DE SEMANA

15. ¿ Tiene Vd. alguna vez disnea caminando aprisa en un terreno llano o subiendo una pequeña cuesta?	No	0 ☐	32
	Yes	1 ☐	
16. ¿ Tiene Vd. disnea caminando con otras personas a paso ordinario en un terreno llano?	No	0 ☐	33
	Yes	1 ☐	
17. ¿ Tiene Vd. que detenerse por que se ahoga caminando a su paso usual en un terreno llano?	No	0 ☐	34
	Yes	1 ☐	
18. ¿ Tiene Vd. disnea al lavarse o al vestirse?	No	0 ☐	35
	Yes	1 ☐	

LOS LUNES

Do not code questions 19-22 for ex-workers.

19. ¿ Tiene Vd. alguna vez disnea caminando aprisa en un terreno llano o subiendo una pequeña cuesta?	No	0 ☐	36
	Yes	1 ☐	
20. ¿ Tiene Vd. disnea caminando con otros personas a paso ordinario en un terreno llano?	No	0 ☐	37
	Yes	1 ☐	
21. ¿ Tiene Vd. que detenerse por que se ahoga caminando a su paso usual en un terreno llano?	No	0 ☐	38
	Yes	1 ☐	
22. ¿ Tiene Vd. disnea al lavarse o al vestirse?	No	0 ☐	39
	Yes	1 ☐	

ANTECEDENTES RESPIRATORIOS

23. ¿ Ha tenido Vd. durante los ultimos 3 años alguna enfermedad del pecho que le haya impedido a trabajar o le haya obligado a permanecer en casa, en cama, o en el hospital?		0 ☐	40
	No	1 ☐	

24. Si la respuesta es afirmativa, pregunta detalles de cada enfermedad:

Año	Duración de la enfermedad		Aumento de la expectoración		Diagnostico del médico
	Menos de l semana	1 Semana o más	Si	No	

Qué hospitales? _____

_____

_____

Code from question 24, the number of illnesses lasting more than one week with increased phlegm. ☐ 41

ANTECEDENTES PATOLOGICOS

25 ¿ Ha tenido Vd. alguna vez

	Si	*No*	*Edad*
a. Bronquitis	____	____	____ a ____
b. Pneumonía	____	____	_____
c. Pleuresía	____	____	_____
d. Tuberculosis pulmonar	____	____	____ a ____
e. Asma bronquial	____	____	____ a ____
f. Otras enfermedades del pecho	____	____	_____

26. Si la respuesta a 25f es afirmativa, especifique: _____

_____

Code later

a. - None -		0 ☐	42
b. Bronchitis *only* (non recurrent)		1 ☐	
c. Bronchitis *only* (recurrent)		2 ☐	
d. Bronchitis *(non recurrent)* + *other chest diseases*		3 ☐	
e. Bronchitis *(recurrent)* + *other chest diseases*		4 ☐	
f. Bronchial asthma *(not at present)*		5 ☐	
g. Bronchial asthma *(still present)*		6 ☐	

27. ¿ Otras enfermedades o operaciones?

No	0 ☐	43
Yes	1 ☐	

Si afirmativa, pregunta detalles:

Hospital _____

Duración en hospital _____

Duración de la incapacidad _____

Complicaciones _____

28. ¿ Han tenidos algunos de sus parientes una o otra de las enfermedades siguientes?
Incluye solamente sus padres, sus hermanos y sus hijos.

No	0 ☐	44
Asma Bronquial	1 ☐	
Asma, eczema o alergia (fiebre del heno)	2 ☐	
Bronquitis	3 ☐	
Bronquitis y pneumonía	4 ☐	

29. ¿ Ha trabajado algún con asma o bronquitis con el algodon/lino o cañamo?

No	0 ☐	45
Yes	1 ☐	

30. ¿ Ha trabajado algún sin asma o bronquitis con el algodon/lino o cañamo?

No	0 ☐	46
Yes	1 ☐	

31. HISTORIA PROFESIONAL

Dé amplios detalles sobre los periodos que ha estado trabajando en minas de carbón y otros minas, fundiciones, fabricas de cerámica, lino, cañamo, amianto y otros ocupaciones expuestas a polvos y cualquier otra exposicion a gases irritantes o vapores químicos.

Año	Domicilio	Industria	Tipo de trabajo	Tipo de fabrica

Code from Occupational History (Question 31).

32. Exposición en la industria de algodon/lino/cañamo.

No	0 ☐	47
Algodon	1 ☐	
Cañamo	2 ☐	
Lino	3 ☐	
Vegetales mistas	4 ☐	
Otras mistas	5 ☐	

33. Edad de la primera exposición en la industria de algodon/lino/cañamo.

<20	0 ☐	48
20-24	1 ☐	
25-29	2 ☐	
30-34	3 ☐	
35-39	4 ☐	
40+	5 ☐	

34. Numero de años del exposicion en la industria de algodon/lino/cañamo.

No	0 ☐	49
<1 año	1 ☐	
1-4	2 ☐	
5-9	3 ☐	
10-19	4 ☐	
20+	5 ☐	

35. Numero de años desde de la ultima exposición en la industria de algodon/lino/cañamo.

No	0 ☐	50
<1 año	1 ☐	
1-4	2 ☐	
5-9	3 ☐	
10-19	4 ☐	
20+	5 ☐	

36. Numero de años del exposición en occupaciones con polvo, gases irritantes o vapores químicos.

No	0 ☐	51
<1 año	1 ☐	
1-4	2 ☐	
5-9	3 ☐	
10-19	4 ☐	
20+	5 ☐	

CONSUMO DE TABACO

37. ¿ Fuma Vd.?
Considere afirmativa la respuesta si el sujeto ha fumado de manera regular hasta hace 1 mes.

No	0 ☐	52
Yes	1 ☐	

Si la respuesta a 37 es negativa:

38. ¿ Ha fumado Vd. alguna vez?
Considere negativa la respuesta si el sujeto ha fumado menos de un cigarrillo al día o menos de 28g (1 onza) de tabaco al mes durante un tiempo inferior a 1 año.

No	0 ☐	53
Yes	1 ☐	

39. Si la respuesta a 37 o 38 es afirmativa, complete con cifras los espacios siguientes.

	AHORA		ANTERIOR	
	Cantidad	Desde	Cantidad	Desde
Cigarrillos al día (Valor medio incluyendo los fines de semana)				
Gramos de tabaco al día (Llados a mano)				
Gramos de tabaco al día (pipa)				
Cigarros a la semana (Tamaño grande)				
Cigarros a la semana (Tamaño pequeño)				

Code later

Record weekly average consumption in grams.

1 gram = 1 cigarette equivalent

None	0 ☐	54
7 — 49 grams/week	1 ☐	
50 — 104 grams/week	2 ☐	
105 — 168 grams/week	3 ☐	
169 + grams/week	4 ☐	

40 ¿ Edad a la que empezó de fumar de manera regular? _____

41. ¿ Edad a la que dejó de fumar de manera regular? _____

42. ¿ Si ha dejado de fumar o fuma menos, dé las

razones de ello: _____

_____

Code later

Number of years of regular smoking:

None	1	☐	55
<1 year	1	☐	
1-4	2	☐	
5-9	3	☐	
10-19	4	☐	
20+	5	☐	

Years since smoking was stopped:

None	0	☐	56
<1 year	1	☐	
1-4	2	☐	
5-9	3	☐	
10-19	4	☐	
20+	5	☐	

LUNG FUNCTION TESTS

1. Name _____

2. Serial number _____

3. Standing height _____

4. Weight _____

Vital Capacity

Instrument number _____ Date _____ Time _____

1. _____

2. _____

3. _____

4. _____

FORCED EXPIRATORY VOLUME (1.0)

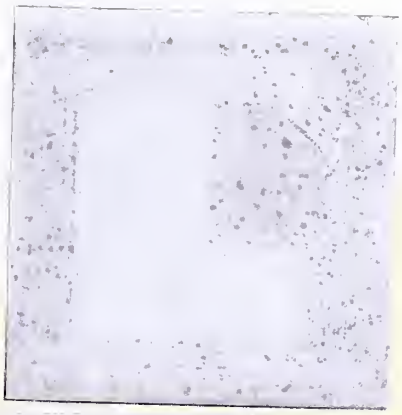
DATE			
TIME			
CONDITION			
	1.		
	2.		
	3.		
	4.		
	5.		
MEAN OF 2 HIGHEST			
INSTRUMENT NUMBER			

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

James Sparks 8-23-82
Yale U Pierce Fndn Lab, 290 Congress Ave, New Haven

NAME AND ADDRESS DATE